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(54) Title: COMPOSITIONS AND METHODS FOR DENDRITIC CELL-BASED IMMUNOTHERAPY

(57) Abstract: Disclosed are immunostimulatory fusion proteins and methods for generating protective DC-induced, T cell-mediated immune responses *in vitro* and *in vivo*. The immunostimulatory fusion proteins comprise a polypeptide antigen component and an immunostimulatory component derived from the intracellular domain of the HER-2 protein. Also disclosed are immunostimulatory compositions comprising dendritic cells pulsed with such an immunostimulatory fusion protein and methods for immunotherapy using the compositions.

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COMPOSITIONS AND METHODS FOR DENDRITIC CELL-BASED IMMUNOTHERAPY

5 Field of the Invention

The present invention relates to an immunostimulatory fusion protein comprising a polypeptide antigen sequence component and a sequence component derived from the intracellular domain of the HER-2 protein which is effective to generate a protective DC-induced, T cell-mediated immune response against the polypeptide antigen; dendritic cells
10 treated with such a composition and methods for immunotherapy using the fusion protein.

Background of the Invention

The HER-2/*erbB*-2 (also called neu) gene encodes a transmembrane glycoprotein of Mr 185,000 (p185) possessing intrinsic tyrosine kinase activity (Akiyama *et al.*, 1986, *Science*
15 232: 1644) and displaying extensive homology to the epidermal growth factor (EGF) receptor (Coussens *et al.*, 1985, *Science* 230: 1132).

Several lines of evidence suggest a link between the amplification of HER-2 and neoplastic transformation. Amplification and overexpression of the HER-2 proto-oncogene occurred in human breast and ovarian cancers and correlated with both poor prognosis and decreased survival in patients (Slamon *et al.*, 1987, *Science* 235: 177; Slamon *et al.*, 1989,
20 *Science* 244: 707).

In experimental systems, tumor antigen specific cytotoxic T lymphocytes (CTL) are the most powerful immunological mechanism for the elimination of tumors. (Greenberg, 1991, *Adv. Immunol.* 49: 281). Therefore, tumor specific antigens (Ag) recognized by CTL are
25 likely to function as tumor rejection Ag, capable of inducing protective immunity *in vivo*.

CTL recognize class I molecules containing peptidic fragments of intracellular proteins that have been transported into endoplasmic reticulum prior to their transfer to the MHC molecule (Germain, 1995, *Ann. NY Acad. Sci.* 754:114; Heemels & Ploegh, 1995, *Annu. Rev. Biochem.* 64:463), while the bulk of class II complexed peptides presented to Th cells are
30 degradation products of exogenous or cell surface proteins that enter the biosynthetic pathway of class II molecules via endocytosis and a subsequent fusion with lysosomes (Cresswell, 1994, *Annu. Rev. Immunol.* 12: 259). CTL are induced when a protein enters the major histocompatibility complex class I ("MHC I" or "class I") pathway of antigen processing. To enter this pathway the protein must be present in the cytosol of an antigen presenting cell
35 (APC). There it is degraded into peptides which are then transported into the endoplasmic reticulum, where they associate with HLA class I molecules. These peptides are then displayed together with the class I molecules on the cell surface and can serve as an inducer and target of class I restricted antigen-specific CTL (Rothbard *et al.*, 1987, *Nature* 326: 881).

The priming of an immune response expands and activates "naive" lymphocytes, *i.e.*,
40 those that have not previously seen a given immunogen such that they become "effector" cells that actively respond. Each naive cell has the potential for seeing one and only one antigenic

epitope, a situation analogous to a key fitting into a lock. Only those cells that recognize their cognate epitope become effector cells.

T-cells can be of the "helper" or "cytotoxic" type. Helper T cells secrete growth factors for lymphoid cells that stimulate the activation and function of B and T cells. The cytotoxic T cells recognize and either directly, or indirectly, kill cells that express a particular antigen. Like B cells, each T cell has receptors specific for one and only one antigenic epitope. T cell receptors recognize fragments of proteins that are displayed on the cell surface by major histocompatibility complexes (MHC). The *in vivo* induction of CTL has typically been accomplished by immunization with live virus or cells (Tanaka, et al., *J. Immunol.*, (1991), 147, 3646-52, Wang, et al., *J. Immunol.*, (1995), 4685-4692). A characteristic of DC, a potent subset of APC, is their ability to trigger *in vivo* responses of naïve CD8⁺ cytotoxic T-lymphocytes (CTL), after being pulsed with antigen (Ridge *et al.* 1998 *Nature* 393:474).

Besides their immature (resting or precursor) form, DC exist in two mature states: activated and superactivated. Activated DC can stimulate CD4⁺ T helper cells, but not CD8⁺ cytotoxic T cells (CTL), while superactivated DC possesses the ability to stimulate CD8⁺ CTL.

Although tumor cells may express protein antigens that are recognized as foreign by the subject, and immune surveillance may limit the growth and spread of some types of tumors, the immune system does not always protect the subject from lethal human cancers. Such tumors may overwhelm the immune system due to rapid growth and spread and/or the tumor cells may evade immune destruction. Proposed mechanisms for such evasion include, but are not limited to, (1) down-regulation of Class I MHC antigens on the surface of tumor cells resulting in little or no complexing of processed tumor peptide antigens with Class I MHC as required for recognition by cytotoxic T lymphocytes (CTL), (2) a lack of activation of CTL due to little or no expression of Class II MHC molecules by tumor cells such that they cannot directly activate tumor-specific CD4⁺ helper T cells (which produce signals likely to be needed for CTL activity), (3) a lack of co-stimulation cell surface markers that provide secondary signals for activation of CD4⁺ helper T cells, and (4) factors produced by tumor cells that suppress anti-tumor responses, such as fas-ligand (Abbas, A.K. *et al.*, Eds., CELLULAR AND MOLECULAR IMMUNOLOGY, 3rd edition, W.B. Saunders Co., 394-405, 1997).

It is therefore desirable to provide a means for eliciting CTL responses against tumor-specific proteins. CTL can be induced either *in vivo* with vaccines or can be generated *in vitro* and then be re-infused into the tumor-bearing organism.

Summary of the Invention

The invention is directed to an immunostimulatory fusion protein which includes a polypeptide or protein antigen sequence component and a sequence component derived from the intracellular domain of the HER-2 protein and is effective to elicit an immune response to the polypeptide or protein antigen sequence component of the fusion protein.

In one aspect, the HER-2 intracellular domain sequence component of the immunostimulatory fusion protein has the sequence presented as SEQ. ID. NO: 25.

In another aspect, the polypeptide or protein component is associated with tumor cells or the causative agent of an infectious disease.

In general, the immunostimulatory fusion protein is produced by translation of a continuous nucleic acid coding sequence. However, the fusion protein may also be produced by chemical coupling.

In one preferred embodiment, the polypeptide or protein component of the fusion protein is the mature HER-2 membrane distal extracellular domain sequence presented as SEQ. ID.

NO: 23. Examples of such fusion proteins and the associated amino acid sequences are:

HER500 (SEQ ID NO: 1), HER500•hGM-CSF (SEQ ID NO: 2), HER500* (SEQ ID NO:3)

and HER500*•rGM-CSF (SEQ ID NO: 4).

The invention provides an immunostimulatory fusion protein composition which can mediate a dendritic cell-induced, T cell-mediated immune response.

In one aspect, the immunostimulatory fusion protein composition comprises a polypeptide or protein antigen sequence component and a sequence component derived from the intracellular domain of the HER-2 protein.

In a related aspect, the immunostimulatory fusion protein composition comprises dendritic cells activated by *in vitro* exposure to an immunostimulatory fusion protein of the invention alone, or in combination with the immunostimulatory fusion protein.

The invention also provides a method of producing superactivated DC, by exposing DC to an immunostimulatory fusion protein of the invention, in a manner effective to result in an cellular immune response to the polypeptide or protein antigen sequence component of the fusion protein. In practicing the method, DC may be exposed to immunostimulatory fusion protein *in vitro* or *in vivo*.

The invention provides methods, and compositions, for use immunotherapy of primary or metastatic cancers that are associated with a particular antigen. DC's are obtained from a human donor, exposed to an immunostimulatory fusion protein of the invention in a manner and for a time effective to result in antigen-loaded superactivated DC. The latter are then administered to a subject who has a cancer associated with expression of the polypeptide or protein component of the immunostimulatory fusion protein, resulting in an immunotherapeutic growth inhibiting response against the primary or metastatic cancer or tumor. In such cases, administration of such superactivated DC may be carried out in combination with co-administration of an immunostimulatory fusion protein to the patient.

In another related approach, the invention provides a method of treating cancer where the cancer is associated with the expression of a particular antigen, by administering an immunostimulatory fusion protein of the invention to a patient diagnosed with the cancer in a manner effective to result in an immune response to the polypeptide or protein antigen sequence component of the fusion protein.

In one exemplary embodiment of these aspects of the invention, the cancer is breast carcinoma, ovarian cancer or colon cancer and the polypeptide or protein antigen sequence component of the immunostimulatory fusion protein is the mature HER-2 membrane distal extracellular domain sequence presented as SEQ. ID. NO: 23.

Brief Description of the Drawings

Figure 1 shows the response of the interleukin-2 (IL-2) secreting mouse MHC class I dependent CD8⁺ T cell hybridoma, B3Z [specific for the chicken ovalbumin "OVA" derived immunodominant peptide SIINFEKL (Jameson et al., 1993, *J. Exp. Med.* 177: 1541)], to various antigens (Ag) presented by syngeneic superactivated dendritic cells (DC). CPM refers to counts per minute; cpm refers to a difference between the absolute cpm for a given test group minus the background cpm value obtained in the absence of the soluble Ag (in the experiment shown the latter was 9,581). The composition of the various antigens is indicated in the figure is described below.

Figure 2A shows the results of an experiment directed to the effect of pre-immunization with Ag-pulsed superactivated DC on the survival of mice up to 42 days after challenge with tumor cells, using "None" (open squares), "HER300*•rGM-CSF" (closed triangles) and HER500*•rGM-CSF (open circles), as the immunizing antigen.

Figure 2B shows the results of another experiment directed to the effect of pre-immunization with Ag-pulsed superactivated DC on the survival of mice up to 77 days after challenge with tumor cells, using "None" (open circles, 7 mice), "HER500*" (open squares, 7 mice), HER500*•rGM-CSF (closed triangles, 8 mice), "HER500" (closed circles, 7 mice), and HER500•hGM-CSF (closed diamonds, 7 mice), as the immunizing antigen.

Figure 3 shows the results of an experiment directed to the effect of post-infection immunization with Ag-pulsed superactivated DC on the survival of mice (10/group) up to 63 days after tumor cell injection, using "None" (closed squares), "HER500*•rGM-CSF" (closed triangles), HER500 (closed diamonds), and "HER500•hGM-CSF" (open circles) as the immunizing antigen.

Detailed Description of the Invention

I. Definitions

Unless otherwise indicated, the terms below have the following meanings:

As used herein, "presentation of soluble protein antigens in the context of major histocompatibility complex class I molecules (MHC I)" means the soluble protein antigen or fragments thereof, are displayed together with major histocompatibility complex class I molecules on the cell surface and can serve as an inducer and target of class I restricted antigen-specific CTL.

As used herein, the term "pulse" means exposure of APC to antigen for a time sufficient to promote presentation of that antigen on the surface of the APC.

As used herein, the term "modified antigen presenting cells" (modified APC) or "modified dendritic cells" (modified DC) refers to a population of APC or DC, respectively, which have been treated (pulsed) *ex vivo* in manner effective to result in an enhanced ability to present antigen in the context of MHC class I relative to APC or DC which have not been so modified.

The term "more effectively" when used herein relative to the presentation of soluble proteins antigens means at least a 2-fold increase in the magnitude of detectable T cell response following presentation of a soluble protein antigen by APC. For example, this means that at

least a 2-fold increase in the magnitude of T cell response is detected following presentation of a given antigen by a designated number of APC relative to magnitude of T cell response obtained when a different or modified antigen is presented by the same number of APC under the same culture conditions and at an equimolar Ag concentration.

5 As used herein, "antigen presenting cells" (APC) are any cells which, after being pulsed with Ag, can activate T-lymphocytes.

As used herein, "dendritic cells", or "DC", are the most potent subset of APCs that are typically large veiled cells which extend dendrites when cultured *in vitro*.

10 As used herein, "activated DC" are mature DC that can stimulate CD4⁺ helper T cells, but not CD8⁺ cytotoxic T cells (CTL).

As used herein, "superactivated DC" are mature DC that can stimulate CD8⁺ cytotoxic T cells (CTL).

As used herein, the term "allostimulatory" means capable of stimulating allogeneic T cells due to differences in MHC molecules expressed on the cell surface.

15 An "antigen" or "Ag" refers to a substance that reacts alone or in the context of MHC molecules with the products of an immune response (*e.g.*, antibodies, T-cell receptors) which have been stimulated by a specific immunogen. Antigens therefore include the specific immunogens giving rise to the response (*e.g.*, antigenic peptides, proteins or polysaccharides) as well as the entities containing or expressing the specific immunogens (*e.g.*, viruses, 20 bacteria, etc.).

As used herein, "immunogen" refers to a substance that is able to stimulate or induce a humoral antibody and/or cell-mediated immune response.

"Ag-loaded DC", include DC and various types of PBMC including professional APC and monocytes/macrophages, which have been exposed to an antigen and activated by the Ag. 25 DC may become Ag-loaded *in vitro*, *e.g.*, by culture *ex vivo* in the presence of a tumor Ag, or *in vivo* by exposure to a tumor antigen.

As used herein, the term "superactivated dendritic cell" refers to DC or DC precursors which have been treated *ex vivo* in such a way that they have an enhanced ability to present antigen in the context of MHC class I relative to the untreated DC.

30 As used herein, the terms "immunostimulatory fusion protein composition" and "antigenic fusion protein composition", may be used interchangeably and refer to a fusion protein of the invention which comprises an antigenic sequence component and a HER-2 intracellular domain sequence component alone and/or DC which have been exposed to such a fusion protein, as further described below.

35 As used herein, "OVA" refers to native ovalbumin; "*" refers to the immunodominant OVA-derived peptide SIINFEKL; "HER500" refers to the recombinant fusion human HER-2 protein consisting of one half of its extracellular portion fused to the 1/4 of its intracellular part; "HER500*" refers to the recombinant fusion protein made of HER500 and the immunodominant OVA-derived peptide SIINFEKL inserted between its extracellular and 40 intracellular components; "HER500*•rGM-CSF" refers to the recombinant fusion protein composed of HER500* and rat granulocyte/macrophage colony-stimulating factor (GM-CSF); "HER500•hGM-CSF" refers to the recombinant fusion protein composed of HER500 and

human GM-CSF; and "HER300*•rGM-CSF" refers to the recombinant fusion human HER-2 protein consisting of one half of its extracellular portion fused to the immunodominant OVA-derived peptide SIINFEKL and rat GM-CSF, as summarized below.

By "protective T cell mediated response" is meant the T cell activity that leads to a slowing or diminution of the growth of cancer cells or a solid tumor, or a reduction in the total number of cancer cells or total tumor burden.

By "cancer or tumor" cell is meant a cell that exhibits a loss of growth control and forms unusually large clones of cells. Tumor or cancer cells generally have lost contact inhibition and may be invasive and/or have the ability to metastasize.

"Tumor antigens" refer to Ag associated with a particular type of cancer or tumor, including tumor-associated Ag and tumor-specific Ag. Examples of tumor antigens are provided below in Section IIA.

As used herein, the term "improved therapeutic outcome" relative to a cancer patient refers to a slowing or diminution of the growth of cancer cells or a solid tumor, or a reduction in the total number of cancer cells or total tumor burden.

As used herein, the term "improved therapeutic outcome" relative to a subject diagnosed as having an infectious disease, refers to a slowing or diminution in the growth of the causative infectious agent within the subject and/or a decrease in, or elimination of, detectable symptoms typically associated with the particular infectious disease.

II. Immune Response to Soluble Polypeptide Antigens

In experimental systems, tumor antigen specific cytotoxic T lymphocytes (CTL) are the most powerful immunological mechanism for the elimination of tumors. CTL can be induced either *in vivo* with vaccines or can be generated *in vitro* and then be re-infused into the tumor-bearing organism. The *in vivo* induction of CTL is typically accomplished by immunization with live virus or cells (Tanaka, *et al.*, *J. Immunol.*, (1991), 147, 3646-52, Wang, *et al.*, *J. Immunol.*, (1995), 4685-4692).

With the exception of a few special viral proteins such as the SV-40 large T antigen and the Hepatitis B surface antigen, injection of isolated or soluble proteins does not result in induction of CTL (Schirmbeck, *et al.*, *Eur. J. Immunol.*, (1993), 23, 1528-34). CTL are induced when a protein enters the major histocompatibility complex class I ("MHC I" or "class I") pathway of antigen processing. To enter this pathway the protein must be present in the cytosol of an antigen presenting cell (APC). There it is degraded into peptides which are then transported into the endoplasmic reticulum, where they associate with HLA class I molecules. These peptides are then displayed together with the class I molecules on the cell surface and can serve as an inducer and target of class I restricted antigen-specific CTL. Physiologically, only proteins that are endogenously synthesized by the APC enter this pathway.

The priming of an immune response expands and activates "naive" lymphocytes, *i.e.*, those that have not previously seen an immunogen to become "effector" cells that actively respond. Each naive cell has the potential for seeing one and only one antigenic epitope, a situation analogous to a key fitting into a lock. Only those cells that recognize their cognate epitope become effector cells.

T-cells can be of the "helper" or "cytotoxic" (cytotoxic) type. Helper T cells secrete growth factors for lymphoid cells that stimulate the activation and function of B and T cells. The cytotoxic T cells recognize and either directly, or indirectly, kill cells that express a particular antigen. Like B cells, each T cell has receptors specific for one and only one antigenic epitope. T cell receptors recognize fragments of proteins that are displayed on the cell surface by major histocompatibility complexes (MHC).

There are two different types of MHC proteins, Class I and Class II, both of which present proteolytically degraded fragments of proteins to T cells. Class I molecules which are expressed on most cells of the body and present fragments of endogenously synthesized proteins to cytotoxic T cells. Class II molecules which are expressed on specialized antigen presenting cells (APCs) such as macrophages, monocytes, dendritic cells and B cells present protein fragments to T helper cells. (Chen, CH and Wu, TC, *J Biomed Sci.*, 5(4):231-52 1998).

In most cases, Class I molecules present foreign proteins synthesized in a cell. For presentation by Class II, the foreign protein either can be synthesized in the cell or taken up by the cell from the outside (*i.e.*, presented in the form of a free protein or peptide). If an antigen is synthesized in a cell and presented by both Class I and Class II molecules, both antibody producing B cells and cytotoxic T cells are produced. However, if an antigen originated outside of a cell and is expressed only by Class II, the specific immune response is largely limited to T helper cells and antibody production. [THE SCIENTIFIC FUTURE OF DNA FOR IMMUNIZATION, American Academy of Microbiology, Robinson, *et al.*, Eds., 1-29, 1997]

Accordingly, the typical response to soluble protein antigens is a Class II mediated response. The present invention represents compositions and methods which allow soluble protein antigens to enter the Class I presentation pathway.

In addition, some progeny of antigen-stimulated T cells do not develop into effector cells, but become memory cells that are capable of surviving for long periods of time in the absence of additional antigenic challenge. Such memory cells are quiescent and do not produce effector molecules unless they are stimulated by antigen. (See, *e.g.*, Abbas, AK *et al.*, Eds. CELLULAR AND MOLECULAR IMMUNOLOGY, W.B. Saunders Co., pages 116-123; 130-134, 1997).

Naïve T cells (or T cells that have not been previously exposed to a given antigen) require only the correct MHC I-restricting molecule to survive, however to expand, they also must be exposed to antigen. In contrast, memory T cells have a lower functional activation threshold that facilitates secondary responses which are more rapid and stronger than that of naïve T cells.

A. Polypeptide Antigens

The present invention is based on the discovery that immunostimulatory compositions comprising a polypeptide antigen component and a sequence component derived from the intracellular domain of the HER-2 protein are effective to generate a protective DC-induced, T cell-mediated immune response against the polypeptide antigen.

Polypeptide antigens of particular interest are those associated with cancer cells, tumors and/or infectious agents.

For example, "tumor-specific antigens" and "tumor-associated antigens" that are characteristic of a particular tissue type, including particular tumor tissues find utility in the immunostimulatory fusion proteins of the invention. Exemplary tumor antigens include, but are not limited to HER-2/neu; prostatic acid phosphatase (PAP); MART-1 (associated with melanoma; Coulie, *et al.*, *J. Exp. Med.* 180:35, 1994; Hawakami, *et al.*, *PNAS* 91:3515, 1994; Bakker, *et al.*, *J. Exp. Med.* 179:1005, 1994); the tumor rejection antigen precursors, MAGE, BAGE and GAGE; NY-ESO (cloned from an esophageal cancer); SART-3 (a squamous cell carcinoma antigen), immunoglobulin antigens specific to particular B-cell lymphomas, tumor-associated antigens such as carcinoembryonic antigen (CEA), p53, c-myc, neural cell adhesion molecule (N-CAM) and polymorphic epithelial mucin (PEM), in addition to any of a number of proteins expressed on tumor cells.

Also of interest are antigens specific to particular infectious agents, e.g., viral agents including, but not limited to human immunodeficiency virus (HIV), hepatitis B virus (HBV), influenza, human papilloma virus (HPV), foot and mouth (coxsackieviruses), the rabies virus, herpes simplex virus (HSV), and the causative agents of gastroenteritis, including rotaviruses, adenoviruses, caliciviruses, astroviruses and Norwalk virus; bacterial agents including, but not limited to *E. coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Neisseria gonorrhoeae*, *Helicobacter pylori*, *Hemophilus influenzae*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Mycobacterium tuberculosis* and *Streptococcus pneumoniae*, fungal agents and parasites such as *Giardia*.

HER-2. An Exemplary Antigen for use in Immunostimulatory Fusion Proteins

Malignant tumors express a number of proteins including molecules belong to the group of transformation-related molecules such as the oncogene HER-2/Neu/ErbB-2.

Likewise, oncogene product peptide antigens have been identified that are common to specific tumor types. These polypeptides find use as reagents that can generally stimulate T-cell responses effective to react with tumors bearing such antigens. The oncogene product peptide antigen, HER-2/neu (Beckmann *et al.*, *Eur. J. Cancer* 28:322, 1992) is associated with human breast and gynecological cancers.

The association of HER-2 overexpression in cancer cells with malignant phenotypes and chemoresistance is consistent with poor clinical outcome for patients with HER-2-overexpressing tumors. The following is a brief summary of HER-2 overexpression associated with various cancers:

Amplification and overexpression of the HER-2 gene is found in 25-30% of primary breast cancers and is associated with a poor clinical outcome. *In vitro* studies of HER-2 overexpression promoted down-regulation of the estrogen receptor (ER) in estrogen-dependent breast tumor cells (Pietras *et al.*, 1995, *Oncogene* 10: 2435), consistent with clinical data that shows HER-2 overexpression is associated with the ER-negative phenotype (Zeillinger *et al.*, 1989, *Oncogene* 4: 109; Adnane *et al.*, 1989, *Oncogene* 4: 1389), and the failure of tamoxifen therapy in patients with HER-2 overexpression (Wright *et al.*, 1992, *Br. J. Cancer* 65:118).

A study on colorectal cancer patients. demonstrated that the level of HER-2 expression correlated with the progression of colorectal cancer, the relapse-free period and postoperative survival time, and could serve as an independent prognostic factor in HER-2-positive colorectal cancers (Kapitanovic *et al.*, 1997, *Gastroenterology* 112: 1103).

5 The expression of HER-2 protein has also been described as an independent prognostic indicator in ovarian (Slamon *et al.*, 1989, *Science* 244: 707) and endometrial cancers (Saffari *et al.*, 1995, *Cancer Res.* 55: 5693).

Overexpression of HER-2 in NIH/3T3 cells resulted in cellular transformation and tumor growth in athymic mice (Di Fiore *et al.*, 1987, *Science* 237: 178; Hudziak *et al.*, 1987, 10 *Proc. Natl. Acad. Sci. USA* 84: 7159). Cellular and animal experiments have shown that the enhanced HER-2 tyrosine kinase activity increased the expression of malignant phenotypes (Hudziak *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84: 7159; Muller *et al.*, 1988, *Cell* 54: 105; Yu & Hung, 1991, *Oncogene* 6:1991; Yu *et al.*, 1993, *Cancer Res.* 53: 891; Zhau *et al.*, 1996, *Prostate* 28: 73). Transgenic mice overexpressing activated c-neu oncogene driven by a 15 mouse mammary tumor promoter, developed synchronously multiple mammary tumors involving the entire glands (Muller *et al.*, 1988, *Cell* 54: 105).

Overexpression of HER-2 was also reported to induce resistance to chemotherapeutic drugs in NSCLC, gastric adenocarcinoma and breast cancers (Tsai *et al.*, 1993, *J. Natl. Cancer Inst.* 85: 897; Tsai *et al.*, 1995, *J. Natl. Cancer Inst.* 87: 682; Paik *et al.*, 1991, *Proc. Am. Assoc. Cancer Res.* 32:291; Wright *et al.*, 1992, *Br. J. Cancer* 65:118). 20

HER-2 peptide and polypeptide antigens can be isolated, synthesized or recombinantly expressed according to methods known in the art. The DNA coding sequence for HER-2/Neu/ErbB-2 may be found at GenBank Accession No. M11730 (human c-erb-B-2 mRNA).

Such isolated HER-2 antigens can be complexed with any of a number of molecules that 25 enhance the immune response to the antigen, as discussed below, either chemically, or as fusion proteins produced recombinantly, according to methods well known in the art.

III. Immunostimulatory Fusion Proteins

The present invention is based on the discovery that immunostimulatory fusion proteins 30 comprising a polypeptide antigen sequence component and a sequence component derived from the intracellular domain of the HER-2 protein are effective to generate a protective DC-induced, T cell- mediated protective immune response against the antigenic component of the fusion protein.

An exemplary intracellular domain of the HER-2 protein is presented herein, however it 35 will be understood that shorter fragments of the exemplary sequence may also exhibit activity. Most importantly, a sequence component derived from the intracellular domain of the HER-2 protein has been demonstrated to contribute to the immunostimulatory activity of fusion proteins comprising such sequence components.

An immunostimulatory fusion protein construct of the invention may also include one or 40 more sequence components selected from the group consisting of GM-CSF, a reporter sequence such as the immunodominant OVA-derived octapeptide SIINFEKL (OVA₂₅₇₋₂₆₄), one or more

peptide signal sequences and a synthetic purification tag, e.g., an added C-terminal amino acid sequence.

Exemplary immunostimulatory fusion protein constructs described herein have a 32 amino acid PAP signal sequence¹, a 3 amino acid mature PAP sequence², a 3 amino acid HER-2 signal sequence³, and either a C-terminal amino acid purification tag sequence of 9 amino acid residues comprising three consecutive alanines and six consecutive histidines, or a C-terminal amino acid tag sequence of 15 amino acid residues comprising glycine, alanine, 4 consecutive prolines, three consecutive alanines, and six consecutive histidines and the features summarized in Table 1, below.

Table 1. Components Of Exemplary HER-2 Fusion Proteins.

| Construct | HER-2 extracellular sequence ⁴ | SIINFEKL (OVA ₂₅₇₋₂₆₄) | HER-2 intracellular sequence ⁵ | GM-CSF |
|-----------------|---|------------------------------------|---|----------------|
| HER500 | + | - | + | - |
| HER500•hGM-CSF | + | - | + | 127 aa (human) |
| HER500* | + | + | + | - |
| HER500*•rGM-CSF | + | + | + | 127 aa (rat) |
| HER300*•rGM-CSF | + | + | - | 127 aa (rat) |

It will be understood that a fusion protein comprising a polypeptide antigen sequence component and a sequence component derived from the intracellular domain of the HER-2 protein are alone effective to generate a protective DC-induced, T cell-mediated protective immune response against the antigenic component of the fusion protein.

Accordingly, the SIINFEKL (OVA₂₅₇₋₂₆₄) sequence, the PAP signal sequence, the mature PAP amino acid sequence, the HER-2 signal sequence, and the C-terminal peptide tag sequence set forth above are not necessary to generate such a response.

The immunostimulatory fusion proteins of the invention may be modified by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known in the art and the selection of the reporter determines the assay format. For example, as detailed in Example 1, the OVA-derived immunodominant octapeptide SIINFEKL (OVA₂₅₇₋₂₆₄) was incorporated into exemplary immunostimulatory HER-2 fusion constructs and antigen presentation of the constructs evaluated. Briefly, the IL-2 secreting mouse T cell hybridoma B3Z, which responds to SIINFEKL (OVA₂₅₇₋₂₆₄), when bound to mouse MHC class I, was stimulated with DC that were pre-pulsed with the HER-2 fusion constructs, and the magnitude of response evaluated by measuring [³H]thymidine incorporation in proliferating IL-2 dependent cells, as an indicator of antigen presentation.

¹ 32 amino acids corresponding to amino acids 1 to 32 of Genebank accession No. NM_001099

² 3 amino acids corresponding to amino acids 33 to 35 of Genebank accession No. NM_001099

³ 3 amino acids corresponding to amino acids 19 to 21 of Genebank accession No. M11730

⁴ 289 amino acids corresponding to amino acids 22 to 310 of Genebank accession No. M11730

⁵ 217 amino acids corresponding to amino acids 1038 to 1254 of Genebank accession No. M11730

Additional examples of fusion proteins for use in practicing the invention include, but are not limited to those which include the sequence of a cancer antigen directly fused to the 217 amino acids of membrane distal intracellular HER-2 domain, without additional linker or signal peptide components. Examples of such fusion proteins include, but are not limited to: a fusion protein comprising 180 amino acids of the human autoimmunogenic cancer/testis antigen, NY-ESO-1 (amino acids 1 to 180 of GenBank Accession No. U87459), fused to the 217 amino acids of membrane distal intracellular HER-2 domain (amino acids 1038 to 1254 of GenBank Accession No. M11730), presented herein as SEQ ID NO: 27, the coding sequence for which is presented as SEQ ID NO: 28); a fusion protein comprising 962 amino acids of the squamous cell carcinoma antigen, SART3-IC (amino acids 1 to 962 of GenBank Accession No. AB020880), fused to the 217 amino acids of membrane distal intracellular HER-2 domain (amino acids 1038 to 1254 of GenBank Accession No. M11730), presented herein as SEQ ID NO: 29, the coding sequence for which is presented as SEQ ID NO: 30).

As known in the art, a recombinant polypeptide may also be produced as a fusion with a heterologous polypeptide, such as a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence is specific to the vector/host cell system used to express the recombinant protein.

Recombinant polypeptides or fusion proteins that include a tag sequence placed for example at the amino- or carboxyl-terminus of the polypeptide are also known in the art. The tag sequence enables the polypeptide to be readily detected using an antibody against the tag and facilitates affinity purification of the polypeptide.

Granulocyte-macrophage colony stimulating factor (GM-CSF), has been included in exemplary fusion constructs of the invention. GM-CSF, a glycoprotein with an apparent molecular weight of about 23-33,000 by SDS-PAGE, is a cytokine that has pleiotropic function both in hematopoiesis as well as in immunology. Human and rat GM-CSF have been shown to bind to cells of the monocyte-macrophage, neutrophil and eosinophil cell lineages. Binding of GM-CSF to high affinity receptors results in rapid internalization and degradation of GM-CSF (Metcalf and Nicola in THE HEMOPOIETIC COLONY-STIMULATING FACTORS, Cambridge University Press, NY (1995)). The immunostimulatory effect of a polypeptide complex consisting essentially of GM-CSF and a polypeptide antigen is further described in USSN 08/579,823 (allowed), expressly incorporated by reference herein. Both human and rat GM-CSF are synthesized with a 17-amino acid hydrophobic leader sequence that is proteolytically cleaved during secretion. The mature polypeptides are 127 amino acids in length, and the sequences may be found at GenBank Accession Nos. NM_000758 and U00620, respectively.

It will be appreciated that the HER-2 extracellular sequence⁶ described herein is an example of an antigenic sequence that may be incorporated into an immunostimulatory fusion protein of the invention. It follows that any of a number of antigens associated with tumor cells or an infectious agent, may similarly be incorporated into an immunostimulatory fusion protein of the invention and be effective to generate a protective DC-induced, T cell-mediated

⁶ 289 amino acids corresponding to amino acids 22 to 310 of Genebank accession No. M11730

protective immune response against the antigenic component of the fusion protein. Exemplary antigens are further described in Section IIA, above.

Exemplary HER-2 Antigen Compositions

5 An immunostimulatory fusion protein of the invention is made by chemical linkage of the antigenic sequence component to the sequence component derived from the intracellular domain of the HER-2 protein, or by expression of a recombinant and continuous nucleic acid coding sequence which is expressed as a fusion protein. Chemical linkage and/or recombinant protein expression may also be used to incorporate additional peptidic sequences into the fusion
10 protein, e.g., a reporter sequence, a signal peptide sequence and/or a purification tag.

Exemplary human HER-2-derived recombinant and continuous nucleic acid coding sequences which have been expressed as fusion proteins are described below.

The exemplary HER500 construct (SEQ ID NO:6) was produced by expression of a coding sequence including in the 5' to 3' direction: the coding sequence for a 32 amino acid
15 PAP signal sequence, the coding sequence for a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, the coding sequence for a 3 amino acid HER-2 signal sequence, the coding sequence for 289 amino acids of the mature HER-2 membrane distal extracellular domain, the coding sequence for 217 amino acids of the HER-2 membrane distal intracellular domain and the coding sequence for three consecutive alanines, and six consecutive histidines.

20 The exemplary HER500•hGM-CSF construct (SEQ ID NO:7) was produced by expression of a coding sequence including in the 5' to 3' direction: the coding sequence for a 32 amino acid PAP signal sequence, the coding sequence for a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, the coding sequence for a 3 amino acid HER-2 signal sequence, the coding sequence for 289 amino acids of mature HER-2 membrane distal
25 extracellular domain, the coding sequence for 217 amino acids of the HER-2 membrane distal intracellular domain, an Ala Ala linker, the coding sequence for mature human GM-CSF (127 residues), and the coding sequence for glycine, alanine, four consecutive prolines, three consecutive alanines, and six consecutive histidines.

The exemplary HER500* construct (SEQ ID NO:8) was produced by expression of a coding sequence including in the 5' to 3' direction: the coding sequence for a 32 amino acid
30 PAP signal sequence, the coding sequence for a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, the coding sequence for a 3 amino acid HER-2 signal sequence, the coding sequence for 289 amino acids of mature HER-2 membrane distal extracellular domain, an Ala linker, the coding sequence for the OVA-derived immunodominant octapeptide
35 SIINFEKL (OVA₂₅₇₋₂₆₄), the coding sequence for 217 amino acids of the HER-2 membrane distal intracellular domain, three consecutive alanines, and six consecutive histidines.

The exemplary HER500*•rGM-CSF construct (SEQ ID NO:9) was produced by expression of a coding sequence including in the 5' to 3' direction: the coding sequence for a 32 amino acid PAP signal sequence, the coding sequence for a 3 amino acid sequence of the
40 mature PAP protein, an Ala Arg linker, the coding sequence for a 3 amino acid HER-2 signal sequence, the coding sequence for 289 amino acids of mature HER-2 membrane distal extracellular domain, an Ala linker, the coding sequence for the OVA-derived immunodominant

octapeptide SIINFEKL (OVA₂₅₇₋₂₆₄), the coding sequence for 217 amino acids of the HER-2 membrane distal intracellular domain, an Ala Ala linker, the coding sequence for mature rat GM-CSF (127 residues), and the coding sequence for glycine, alanine, four consecutive prolines, alanine, and six consecutive histidines.

5 The exemplary HER300*•rGM-CSF construct (SEQ ID NO:10) was produced by expression of a coding sequence including in the 5' to 3' direction: the coding sequence for a 32 amino acid PAP signal sequence, the coding sequence for a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, the coding sequence for a 3 amino acid HER-2 signal sequence, the coding sequence for 289 amino acids of mature HER-2 membrane distal
10 extracellular domain, an Ala linker, the coding sequence for the OVA-derived immunodominant octapeptide SIINFEKL (OVA₂₅₇₋₂₆₄), an Ala linker, the coding sequence for mature rat GM-CSF (127 residues), and the coding sequence for glycine, alanine, four consecutive prolines, alanine, and six consecutive histidines.

Methods for production of immunostimulatory fusion proteins by chemical linkage of
15 an antigenic sequence to a sequence derived from the intracellular domain of the HER-2 protein include conventional coupling techniques known in the art. In constructs which also include one or more added peptidic sequences, chemical linkage is also accomplished using conventional coupling techniques known in the art. For example, the peptides can be coupled using a dehydrating agent such as dicyclohexyl-carbodiimide (DCCI) to form a peptide bond between the
20 two peptides. Alternatively, linkages may be formed through sulfhydryl groups, epsilon amino groups, carboxyl groups or other reactive groups present in the polypeptides, using commercially available reagents. (Pierce Co., Rockford, IL).

IV. Production of recombinant fusion proteins

25 The invention includes immunostimulatory fusion proteins produced using recombinant techniques. Such an immunostimulatory fusion protein may be produced by any of a number of methods routinely employed by those of skill in the art.

Unless otherwise indicated, all terms used herein have the same meaning as they would to one skilled in the relevant art. Practitioners are particularly directed to Sambrook *et al.*
30 (1989) MOLECULAR CLONING: A LABORATORY MANUAL (Second Edition), Cold Spring Harbor Press, Plainview, N.Y. and Ausubel FM *et al.* (1989) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., for definitions and techniques routinely used by those of skill in the art.

The fusion proteins may be produced by culturing recombinant prokaryotic or
35 eukaryotic host cells comprising nucleic acid sequences encoding the fusion protein under conditions promoting expression of the fusion proteins, followed by recovery of the fusion protein from the host cells or the cell culture medium.

The nucleic acid encoding sequence an immunostimulatory fusion protein of the invention is inserted into any one of a variety of expression vectors for expressing a
40 polypeptide, as long as it is replicable and viable in the host. In general, the nucleic acid coding sequence is inserted into an appropriate restriction endonuclease site or site(s) using routine techniques. Such procedures and related sub-cloning procedures are deemed to be

within the scope of those skilled in the art. The vector may comprise regulatory sequences, including for example, non-coding sequences, such as introns and control elements, *i.e.*, promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host and/or in a vector or host environment in which the immunostimulatory fusion protein coding sequence is not normally expressed, operably linked to the coding sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, are commercially available, examples of which are described in Sambrook, *et al.*, (*supra*).

The present invention also relates to host cells which have been genetically engineered to contain a vector effective to express an immunostimulatory fusion protein of the invention by recombinant techniques. Host cells are genetically engineered (*i.e.*, transduced, transformed or transfected) with an appropriate vector which may be, for example, a cloning or expression vector. The vector may take the form of a plasmid, a viral particle, a phage, *etc.* The culture conditions, such as temperature, pH and the like, are those routinely used for the host cell selected for expression, and will be apparent to those skilled in the art.

Methods of introducing nucleic acids into cells for expression of heterologous proteins are also known to the ordinarily skilled artisan. Examples include calcium phosphate transfection, DEAE-Dextran mediated transfection, electroporation, nuclear microinjection, bacterial protoplast fusion with intact cells, and the use of polycations, *e.g.*, polybrene or polyornithine. General aspects of transformation involving mammalian cells have been described in U.S. Patent No. 4,399,216, and Keown *et al.*, *Methods in Enzymology*, 185:527-537 (1990), both of which are expressly incorporated by reference herein.

Suitable host cells for cloning or expressing an immunostimulatory fusion protein of the invention include prokaryote, yeast, insect and higher eukaryotic cells. Suitable prokaryotes include but are not limited to eubacteria, such as gram-negative or gram-positive organisms, for example, *E. coli*.

Suitable host cells for the expression of a glycosylated immunostimulatory fusion protein of the invention are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. The selection of the appropriate host cell is deemed to be within the skill in the art.

A process for producing such an immunostimulatory fusion protein comprises culturing host cells under conditions suitable for expression of the fusion protein and recovering the fusion protein from the cell culture. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in MAMMALIAN CELL BIOTECHNOLOGY: A PRACTICAL APPROACH, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.*, *supra*. More specifically, techniques for expression in the Baculovirus system are described in Engelhard EK *et al. Proc. Nat. Acad. Sci.* 91:3224-3227, 1994, expressly incorporated by reference herein.

Host cells transformed with nucleotide sequences encoding an immunostimulatory fusion protein of the invention may be cultured under conditions suitable for the expression and recovery of the encoded protein from the cell culture. The protein produced by a recombinant

cell may be secreted, membrane-bound, or contained intracellularly depending on the particular sequence and/or the vector used.

As understood by those of skill in the art, an expression vector containing a polynucleotide encoding an immunostimulatory fusion protein of the invention can be designed with signal sequences which direct secretion of the modified immunostimulatory fusion protein through a prokaryotic or eukaryotic cell membrane.

Example 1 details the construction of exemplary immunostimulatory HER-2 fusion proteins, the nucleic acid and deduced amino acid sequences of which are presented in Table 2.

Expression vectors comprising the coding sequence for various HER-2 fusion proteins were used to transfect mammalian 293-EBNA cells and insect SF21 cells. Once constructed, expressed and purified, HER2 fusion proteins containing either rat GM-CSF or human GM-CSF were tested for GM-CSF bioactivities in appropriate assays, routinely employed by those of skill in the art. Both insect and mammalian cell-derived fusion proteins exhibited GM-CSF activity, as evidenced by their ability to support growth of GM-CSF dependent cell lines. Similarly, the presence of HER-2 was verified using HER-2-specific monoclonal antibodies in both Western blot analysis and in an ELISA test, according to methods well-known in the art.

While the foregoing description describes particular embodiments of the present invention, it will be appreciated that persons skilled in the art can substitute the coding sequence for various antigens, and may use different vectors and cell lines for expression, according to known methods and thereby prepare immunostimulatory fusion protein compositions in accordance with the principles described herein.

Whether produced by chemical coupling or by expression of a continuous coding sequence as a recombinant fusion protein, DC may be exposed to an immunostimulatory fusion protein of the invention and be presented by such DC in the context of MHC I, resulting in a cellular immune response to the fusion protein.

V. Generation Of Activated Dendritic Cells (DC)

A. Isolation and Characterization of DC Precursors and DC

Human dendritic cell precursors (DC precursors) may be obtained from any of a number of sources including but not limited to peripheral blood, cord blood, bone marrow and lymphoid organs.

DC precursors isolated and enriched by any of a number of methods known in the art will result in a DC precursor population effective for carrying out the methods of the present invention.

In a preferred approach, DC precursors are obtained from peripheral blood. In this approach, peripheral blood mononuclear cells (PBMC) are collected from healthy donors by standard leukapheresis and DC precursors isolated, for example, by either a one-step or a successive two-step buoyant density centrifugation using buoyant density solution BDS 77 or BDS 77 and 65, respectively (Dendreon Corp.), as described in co-owned USSN 60/168,991 (0021).

DC precursors may be obtained from a healthy subject or a subject known to be suffering from a disease associated with the expression of a particular antigen. Such DC precursors may be allogeneic or autologous.

Once DC precursors are obtained, they are cultured under appropriate conditions and for a time sufficient to expand the cell population and maintain the DC's in a state for optimal antigen uptake, processing and presentation.

In one preferred approach to culture of DC precursors, DC are generated from such DC precursors by culture *ex vivo* in serum free or protein-free medium for 40 hours, in the absence of exogenously added cytokines, as detailed in co-owned USSN 60/158,618. Briefly, DC precursors are cultured in teflon bags (American Fluoroseal) at a density of 1×10^7 per ml in Aim V medium supplemented with 2mM glutamine in a humidified incubator at 37°C under 5% CO₂ for 40 hours. During the culture period DC precursors are pulsed with Ag.

Preferred aspects of DC isolation and culture include the use of culture medium lacking exogenously supplied cytokines and culture under serum-free conditions in a manner effective to result in the generation of Ag-loaded superactivated DC.

The purity of DC in this fraction may be quantified using, for example, flow cytometry (*i.e.*, FACS) analysis for phenotypic characterization as further described in co-owned USSN 60/158,618, together with functional characterization. Cell surface phenotype analysis is carried out using samples consisting of approximately $1-3 \times 10^7$ cells, which are incubated in 10% normal mouse serum in PBS for 10 min., washed in PBS and resuspended in 250-750µl PBS. The cell suspension is then dispensed at 30µl/well into round-bottom 96-well plates. FITC-, PE-, and PerCP-conjugated mAb are added at 10µl/well and cells are incubated for 20 min. in the dark on ice. Cells are then washed with 200µl/well of PBS and resuspended in 400µl/well in PBS, then analyzed by FACScan (Becton Dickinson) using cells labeled with isotype-matched control Ab as a negative control. Preferred functional characteristics of mature DCs include the acquisition of allostimulatory and Ag-presenting abilities.

Ag-loaded superactivated DCs have already processed an Ag and have the ability to present the Ag to the immune cells and quickly generate Ag-specific immune responses, e.g., CTL-mediated T cell responses to tumor antigens.

According to another aspect of the invention, DC's can be preserved by cryopreservation either before or after exposure to a HER-2 fusion protein of the invention. Exemplary methods for cryopreservation are further described in co-owned USSN 60,168,991. For small scale cryopreservation, cells can be resuspended at $20-200 \times 10^6$ /ml in precooled 5% human serum albumin (HAS) (Swiss Red Cross). An equal volume of 20% dimethylsulfoxide (DMSO) in the above HAS solution was then added dropwise. The mixture is aliquoted in cryovials at 1ml/vial and frozen at -80°C in a cryochamber (Nalgene) overnight. The vials are transferred to a liquid nitrogen tank in the morning. For large scale cryopreservation, cells can be resuspended at $30-600 \times 10^6$ /ml in AIM V. An equal volume of 20% AIM V is then added gradually. The mixture is frozen in freezing containers (Cryocyte, Baxter) at 20 ml/bag using a rate-controlled freezing system (Forma).

B. Evaluation of Antigenicity of Fusion Proteins

In Vitro Antigen Presentation

An antigen presentation assay may be used to evaluate the antigen presenting ability of various immunostimulatory fusion proteins. An exemplary assay is described in Example 1, wherein the IL-2 secreting mouse T cell hybridoma B3Z, which responds to the mouse MHC class I (H2-K^b) bound OVA-derived peptide SIINFEKL (OVA₂₅₇₋₂₆₄; Jameson et al., 1993, *J. Exp. Med.* 177: 1541), is stimulated with various DC that have been pre-pulsed with engineered HER-2 fusion proteins, and the magnitude of response is evaluated by measuring [³H]thymidine incorporation in proliferating IL-2 dependent cells.

The assay format described herein may be used to evaluate antigen presentation using a titration of the antigen together with a fixed number of APC.

B. In vivo assays in animal models

An immunostimulatory fusion protein of the invention may be evaluated *in vivo* in animal models. In such cases, pre-immunization of animals with the immunostimulatory fusion protein composition, or superactivated DC treated *ex vivo* with the composition is effective to suppress *in vivo* growth of tumors or an infectious agent.

In one example of this approach, pre-immunization of mice with activated DC pulsed *ex vivo* with the HER-2 fusion protein composition, HER500*•rGM-CSF (SEQ ID NO:4), which has an antigenic component consisting of 289 amino acids derived from the extracellular domain of HER2 fused to 217 amino acids derived from the intracellular domain of HER2, suppressed the *in vivo* growth of HER-2-expressing autologous tumors in mice (Example 2). In another approach, animals, *e.g.*, mice, are inoculated with a particular infectious agent or tumor-forming cells, then treated with an immunostimulatory fusion protein of the invention and evaluated for the ability of the antigenic composition to suppress *in vivo* growth of the infectious agent or an established tumor.

Example 3 illustrates this approach, in that post-infection injection of mice with activated DC pulsed *ex vivo* with the HER-2 fusion protein compositions, HER500*•rGM-CSF (SEQ ID NO:4), HER500*•hGM-CSF (SEQ ID NO:2), HER500* (SEQ ID NO:3), and HER500 (SEQ ID NO:1), increased the survival time for mice previously inoculated with HER-2-expressing autologous tumor cells.

The results of the animal studies confirm that in order to generate an effective anti-tumor response, an immunostimulatory fusion protein of the invention must comprise antigenic sequence component and a sequence component derived from the intracellular domain of HER-2.

VI. Compositions and Methods for Immunotherapy and Cancer Therapy

The present invention provides immunostimulatory fusion protein compositions that are able to effectively present antigen for the induction of both CD8⁺ CTL-mediated as well as CD4⁺ Th cell proliferative responses.

As such, the immunostimulatory fusion protein compositions of the present invention are universally useful and can be employed in a wide range of immunotherapeutic,

immunoprophylactic and cancer therapeutic applications involving generation of primary and secondary immune responses.

The invention also provides modified soluble polypeptide or protein antigens presented in the context of MHC Class I.

5 In a preferred embodiment, immunization with a modified soluble protein antigen of the invention results in an MHC Class I-mediated cellular immune response to an antigen which would not elicit a cellular immune response of the same magnitude, if provided in an unmodified form.

10 Immunization with such a modified soluble protein antigen results in an MHC Class I-mediated cellular immune response which is greater in magnitude and accordingly provides greater protection than a cellular immune response to the same antigen if provided in an unmodified form.

In one preferred embodiment, the invention provides an immunostimulatory composition comprising DCs exposed *ex vivo* to an immunostimulatory fusion protein comprising a polypeptide antigen sequence component and a sequence component derived from the intracellular domain of the HER-2 protein, as described above, which is able to more effectively induce T-cell responses, than a composition comprising the polypeptide antigen alone.

20 An immunostimulatory composition comprising DCs exposed *ex vivo* to an immunostimulatory fusion protein of the invention alone, or in combination with the immunostimulatory fusion protein finds utility in immunotherapy of a subject and can function as a vaccine.

In a related aspect, the invention includes a method of immunizing a subject against a polypeptide antigen associated with a particular type of cancer or infectious disease. The method includes exposing or pulsing DC with an immunostimulatory fusion protein composition, as further described below.

30 In practicing the methods of the invention, the exposing step can be carried out *in vitro* (*ex vivo*), *in vivo* or both *in vitro* and *in vivo*. For example, an immunostimulatory fusion protein of the invention may be directly injected into a subject or DC may be exposed to the immunostimulatory fusion protein *in vitro* in a manner effective to induce cell-surface presentation of the antigenic component of the fusion protein and the pulsed DC returned to the subject.

35 An antigenic composition comprising an immunostimulatory fusion protein alone or in combination with DC stimulated by *in vitro* exposure to the immunostimulatory fusion protein can be used, for example, in direct *in vivo* administration, *ex vivo* somatic therapy, *in vivo* implantable devices or in *ex vivo* extracorporeal devices.

40 It will be understood that any of a number of methods may be used to pulse DC with an immunostimulatory fusion protein of the invention, to make them effective to present antigen in the context of MHC I. The experiments detailed herein demonstrate that activation of DC by exposure to immunostimulatory fusion proteins facilitates processing of the antigenic component of the fusion protein through the "endogenous" class I pathway such that antigens

are presented in association with MHC class I molecules, and accordingly are able to activate CD8⁺ CTL.

From the foregoing, it will be appreciated that the invention provides compositions and methods having the unique feature that processing of soluble protein antigens occurs through the MHC class I, as opposed to class II, pathway.

VII. Therapeutic Applications

A. Exposing DC *ex vivo* To Immunostimulatory Fusion Protein Compositions

The invention is based on the discovery that DC can be exposed to an immunostimulatory fusion protein composition, either *in vitro* (*ex vivo*), or *in vivo* in a subject, resulting in a protective T cell mediated response against the antigenic component of the fusion protein.

DC are treated *in vitro* (*ex vivo*) with an immunostimulatory fusion protein composition, followed by administration to a subject. The subject may be the same individual from whom the DC were obtained (autologous transplantation) or a different individual (allogeneic transplantation). In allogeneic transplantation, the donor and recipient are matched based on similarity of HLA antigens in order to minimize the immune response of both donor and recipient cells against the other.

A subject may be treated with an immunostimulatory fusion protein composition of the invention alone or in combination with a therapeutic regimen typically used for the condition under treatment, e.g. radiation therapy and/or chemotherapy for the treatment of cancer.

In general, DC precursors are obtained, cultured under serum-free conditions in medium lacking exogenously supplied cytokines, as described above, followed by *in vitro* (*ex vivo*) exposure of the DC to an immunostimulatory fusion protein composition of the invention followed by re-infusion of the activated DC into the subject.

Re-infused *ex vivo* immunostimulatory fusion protein composition-treated DC provide a means for rapid generation of an immune response to the antigenic component of the fusion protein.

B. *In vivo* Administration of an Immunostimulatory Fusion Protein Composition

In another aspect, the invention is directed to methods of treating a subject by *in vivo* administration of an immunostimulatory fusion protein composition of the invention.

In one embodiment, the subject has a type of cancer which expresses a tumor-specific antigen. In accordance with the present invention, an immunostimulatory fusion protein may be made which comprises a tumor-specific antigen sequence component and a sequence component derived from the intracellular domain of HER-2. In such cases, DC pulsed *ex vivo* with this immunostimulatory fusion protein are administered to a subject, alone or in combination with the fusion protein, resulting in an improved therapeutic outcome for the subject, evidenced by, e.g., a slowing or diminution of the growth of cancer cells or a solid tumor which expresses the tumor-specific antigen, or a reduction in the total number of cancer cells or total tumor burden.

In a related embodiment, the subject has been diagnosed as having a viral, bacterial, fungal or other type of infection, which is associated with the expression of a particular antigen, e.g., a

viral antigen. In accordance with the present invention, an immunostimulatory fusion protein may be made which comprises a sequence component consisting of the antigen, e.g., an HBV-specific antigen, together with a sequence component derived from the intracellular domain of HER-2. In such cases, DC pulsed *ex vivo* with the immunostimulatory fusion protein are administered to a subject, alone or in combination with the fusion protein, resulting in an improved therapeutic outcome for the subject as evidenced by a slowing in the growth of the causative infectious agent within the subject and/or a decrease in, or elimination of, detectable symptoms typically associated with the particular infectious disease.

In either situation, *in vivo* administration of an immunostimulatory fusion protein of the invention to a subject provides a means to generate a protective DC-induced, T cell-mediated immune response to the antigen in the subject, dependent upon (1) the antigenic composition administered, (2) the duration, dose and frequency of administration, and (2) the general condition of the subject.

In one example, the subject has a HER-2-expressing cancer and administration of an immunostimulatory fusion protein composition of the invention which includes HER2 as the antigenic component of the fusion protein provides a means to improve the therapeutic outcome of the subject. In this embodiment, the immunostimulatory HER-2 composition is administered to the subject in a manner effective to result in a cellular immune response to HER-2-expressing cancer cells in the subject.

C. Treating Patients

Effective delivery of the immunostimulatory fusion protein composition is an important aspect of the invention. In accordance with the invention, such routes of delivery include, but are not limited to, various systemic routes, including parenteral routes, e.g., intravenous (IV), subcutaneous (SC), intraperitoneal (IP), and intramuscular (IM) injection.

It will be appreciated that methods effective to deliver an immunostimulatory fusion protein to dendritic cells or to introduce an immunostimulatory fusion protein or DC composition in close proximity to antigen-expressing cells are also contemplated.

In one preferred embodiment, the immunostimulatory composition is a fusion protein, contained in a pharmaceutically acceptable carrier, and delivered by the intravenous route. In a further aspect of this embodiment, the immunostimulatory fusion protein composition is administered at regular intervals for a short time period, e.g., in bi-weekly intervals for two months or less. However, in some cases the fusion protein composition is administered intermittently over a longer period of time.

Typically, one or more doses of the immunostimulatory fusion protein are administered, generally at bi-weekly intervals for a period of about two months. Preferred doses for administration by the IV, SC or IM route are from about 5 µg/kg per patient to about 5 mg/kg per patient.

In another preferred embodiment, the immunostimulatory composition comprises DC exposed *ex vivo* to an immunostimulatory fusion protein, contained in a pharmaceutically acceptable carrier, and delivered by the IV, SC or IM route.

In one aspect of this embodiment, the immunostimulatory fusion protein composition comprises from 10^7 to 10^{11} DC, which have been exposed to from 100 ng/ml to 1 mg/ml of a given immunostimulatory fusion protein, in a manner effective to generate Ag-loaded DC as described in Examples 2 and 3. Doses of about 10^7 to 10^{11} DC are then administered to the subject by intravenous or SC or IM injection according to established procedures for a short time period, *e.g.*, at bi-weekly intervals for 2 months or less. However, in some cases the immunostimulatory fusion protein composition is administered intermittently over a longer period of time.

Typically, one or more doses of the immunostimulatory fusion protein composition are administered, generally at regular intervals for a period of about 2 months. In general, the method comprises administering to a subject, in a suitable pharmaceutical carrier, an amount of an immunostimulatory fusion protein or DC composition effective to result in an improved therapeutic outcome for the subject under treatment.

It follows that the immunostimulatory fusion protein or DC composition may be administered in any convenient vehicle, which is physiologically acceptable. Such an immunostimulatory fusion protein or DC composition may include any of a variety of standard physiologically acceptable carrier employed by those of ordinary skill in the art. Examples of such pharmaceutical carriers include, but are not limited to, saline, phosphate buffered saline (PBS), water and Ringer's solution. It will be understood that the choice of suitable physiologically acceptable carrier will vary dependent upon the chosen mode of administration.

Sustained release compositions are also contemplated within the scope of this application. These may include semipermeable polymeric matrices in the form of shaped articles such as films or microcapsules.

In preferred applications of the method, the subject is a human subject. The subject may also be a cancer patient, in particular a patient diagnosed as having a cancer which expresses a particular cancer-specific or cancer-associated antigen, and the patient may or may not be under with chemotherapy and/or radiation therapy.

It will be understood that the effective *in vivo* dose of an immunostimulatory fusion protein or DC composition of the invention will vary according to the frequency and route of administration as well as the condition of the subject under treatment. Accordingly, such *in vivo* therapy will generally require monitoring by tests appropriate to the condition being treated and a corresponding adjustment in the dose or treatment regimen in order to achieve an optimal therapeutic outcome.

D. Monitoring Treatment

The efficacy of a given therapeutic regimen involving the methods described herein, may be monitored, *e.g.*, by monitoring the induction of a CTL response, a helper T-cell response, and/or the antibody response to the antigenic component of the fusion protein in peripheral blood using methods well known in the art in addition to monitoring the status of the cancer and the biological condition of the subject, at various time points following such administration.

In cases where the subject has been diagnosed as having a particular type of cancer, the status of the cancer is also monitored using diagnostic techniques appropriate to the type of cancer under treatment. Similarly, in cases where the subject has been diagnosed as having a particular type of infection, the status of the infection is also monitored using diagnostic techniques appropriate to the type of type of infection under treatment.

The immunostimulatory fusion protein or DC composition treatment regimen may be adjusted (dose, frequency, route, etc.), as indicated, based on the condition of the subject under treatment and the results of the assays described above.

VIII. Utility

The present invention provides immunostimulatory fusion protein compositions that are able to effectively present antigen for the induction of both CD8⁺ CTL-mediated as well as CD4⁺ Th cell proliferative responses.

As such, the immunostimulatory fusion protein compositions of the invention are universally useful and can be employed in a wide range of immunotherapeutic, immunoprophylactic and cancer therapeutic applications involving generation of primary and secondary immune responses.

The immunostimulatory fusion protein compositions of the invention find utility in immunotherapy of cancers which are associated with expression of a particular antigen. For example, the HER-2 fusion protein compositions described herein find utility in immunotherapy of HER-2 expressing tumors, such as, breast carcinoma, ovarian cancer and colon cancer.

The advantages of the present invention include induction of enhanced cellular immunity to isolated or soluble polypeptide or protein antigens by presenting an immunostimulatory fusion protein of the invention to a dendritic cell (DC), resulting in DC activation. As discussed above, such induction is not generally observed using soluble, polypeptide or protein antigens as induction materials. The generation of such activated DC, may be accomplished *in vitro* (*ex vivo*) using autologous or allogeneic DC or may take place *in vivo* following administration of an immunostimulatory fusion protein of the invention to a subject.

All patent and literature references cited in the present specification are hereby expressly incorporated by reference in their entirety.

The following examples illustrate but are not intended in any way to limit the invention.

Example 1

Production Of Exemplary HER-2 Fusion Proteins

In one example, human HER-2 was cloned from a SK-BR3 cell line according to methods known in the art. The stop codon at the 3' end of the sequence was mutated away, and a Not I site inserted in its place, to fuse the HER-2 cDNA to C-terminal tag peptide, rat GM-CSF, or human GM-CSF DNA. GM-CSF DNA was cloned from a PBMC library according to standard methods. A Not I site was inserted at the 5' end of the DNA, and an Xba I cloning site was inserted at the 3' end, along with an in-frame stop codon. PCR-generated cDNA's were

digested with appropriate restriction enzymes and cloned into restriction vectors for transfection into specific mammalian or insect cell lines.

Expression vectors comprising the coding sequence for various HER2 fusion proteins were used to transfect mammalian 293-EBNA cells (Invitrogen) (transient expression) and insect SF21 cells (Clontech, Palo Alto, CA). Fusion protein products were recovered from the tissue culture supernatants, and affinity purified by passage over a metal affinity column, (NTA resin, Qiagen). For HER500*•rGM-CSF, analysis by SDS-PAGE revealed protein bands migrating at 120 kDa and 110 kDa as products from mammalian and insect cells, respectively. The predicted size of the 690 polypeptide backbone is 74877 Da.

Human HER-2-derived proteins were produced as recombinant proteins using the following coding sequences.

The HER500*•rGM-CSF construct (SEQ ID NO: 4) was produced by expression of a coding sequence which included, in the 5' to 3' direction: a 32 amino acid PAP signal peptide, a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, a 3 amino acid HER-2 signal sequence, 289 amino acids of the mature HER-2 membrane distal extracellular domain, an Ala linker, the OVA-derived immunodominant octapeptide SIINFEKL (OVA₂₅₇₋₂₆₄), 217 amino acids of the HER-2 membrane distal intracellular domain, an Ala Ala linker, a 127 amino acid mature rat GM-CSF sequence, and Gly Ala Pro Pro Pro Ala His His His His His His.

The HER300*•rGM-CSF construct (SEQ ID NO: 5) was produced by expression of a coding sequence which included, in the 5' to 3' direction: a 32 amino acid PAP signal sequence, a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, a 3 amino acid HER-2 signal sequence, 289 amino acids of the mature HER-2 membrane distal extracellular domain, an Ala linker, the OVA-derived immunodominant octapeptide SIINFEKL (OVA₂₅₇₋₂₆₄), an Ala linker, a 127 amino acid mature rat GM-CSF sequence, and Gly Ala Pro Pro Pro Pro Ala His His His His His His.

BP8 baculovirus expression vectors (Clontech) comprising the coding sequence for the HER500*•rGM-CSF (SEQ ID NO: 9) or HER300*•rGM-CSF (SEQ ID NO: 10) fusion proteins were used to transfect SF21 cells. Fusion protein products were recovered from tissue culture supernatants, and affinity purified by metal affinity chromatography. Analysis by SDS-PAGE revealed protein bands migrating at 105 kDa for HER500*•rGM-CSF and 60 kDa for HER300*•rGM-CSF.

Once constructed, expressed and purified, HER-2 fusion molecules containing rat GM-CSF or human GM-CSF were tested for GM-CSF bioactivity appropriate assays and the presence of HER-2 was verified using HER-2-specific monoclonal antibodies in both Western blot analysis and in an ELISA test, according to methods well-known in the art.

Evaluation of *in vitro* presentation of HER-2 fusion proteins

The IL-2 secreting mouse T cell hybridoma B3Z, which responds to the mouse MHC class I (H2-K^b) bound OVA-derived peptide SIINFEKL (OVA₂₅₇₋₂₆₄; Jameson et al., 1993, *J. Exp. Med.* 177: 1541), was used to evaluate the antigen presenting ability of HER-2 fusion proteins containing the OVA-derived immunodominant peptide SIINFEKL.

Tissue cultures were maintained in IMDM medium supplemented with 10% FCS, 2 mM L-glutamine, 0.1 mg/ml kanamycin sulfate and 3×10^{-5} M 2-ME (Gibco, Grand Island, NY) at 37°C in a humidified atmosphere containing 5% CO₂ (tissue culture incubator).

Enriched preparations of activated DC were obtained by incubating autologous C57BL/6 spleen cells in tissue culture flasks for 2 h at 37°C, removing non-adherent cells, and culturing the remaining adherent cells for 2 days with 1 µM ionomycin.

An IL-2 secretion assay was performed as described previously [Kruisbeek, 1998, in Coligan *et al.* (eds.) *Current Protocols in Immunology*, Wiley, New York, NY, 1:3.14]. More specifically, 10⁵ hybridoma cells were cultured in 0.2-ml microwells in the presence of 3 x 10⁴ activated DC and various concentrations of HER-2 antigens. One day later, culture supernatants were harvested and tested at 50% concentration for their ability to support the proliferation of 10⁴ HT-2 cells (an IL-2 dependent cell line) for 24 h, as measured by [³H]thymidine incorporation during the final 6 h culture period.

The response of B3Z cells to the HER300*•rGM-CSF and HER500*•rGM-CSF fusion proteins relative to OVA (Grade VII, 99% pure chicken ovalbumin purchased from Sigma, St. Louis, MO), was evaluated *in vitro*. The cell proliferation response indicated as CPM based on 3H thymidine uptake (Fig. 1) indicate that HER300*•rGM-CSF and HER500*•rGM-CSF fusion proteins are more efficient in stimulating B3Z than native OVA itself (about, 10-fold, and > 100-fold, respectively). The 10-fold superiority of HER500*•rGM-CSF over HER300*•rGM-CSF indicates that the enhanced presentation of Ag is correlated with the inclusion of the additional intracellular HER-2 domain derived 217 amino acids in the fusion protein (which are present in HER500*•rGM-CSF but absent in HER300*•rGM-CSF).

Example 2

Prevention of *in vivo* tumor growth by pre-immunization with Ag-pulsed DC

The effect of pre-immunization with HER-2-pulsed activated DC on suppression of *in vivo* growth of HER-2-expressing autologous tumors was evaluated in a murine model.

The mouse tumor cell line E.HER-2 was generated by transfecting EL-4 cells (C57BL/6 mouse strain derived thymoma; ATCC, Rockville, MD) with the full length human HER-2 cDNA according to standard methods.

Human HER-2-derived proteins were produced as recombinant proteins as previously described in Example 1, using the following coding sequences.

The HER500 construct (SEQ ID NO: 1) was produced by expression of a coding sequence which included, in the 5' to 3' direction: a 32 amino acid PAP signal sequence, a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, 3 amino acids of HER-2 signal sequence, 289 amino acids of mature HER-2 membrane distal extracellular domain, 217 amino acids of the HER-2 membrane distal intracellular domain and a C-terminal tag consisting of Ala Ala Ala His His His His His.

The HER500*•hGM-CSF construct (SEQ ID NO: 2) was produced by expression of a coding sequence which included, in the 5' to 3' direction: a 32 amino acid PAP signal sequence, a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, 3 amino acids of HER-2 signal sequence, 289 amino acids of the mature HER-2 membrane distal

extracellular domain, 217 amino acids of the HER-2 membrane distal intracellular domain, an Ala Ala linker, a 127 amino acid mature human GM-CSF sequence and a C-terminal tag consisting of Gly Ala Pro Pro Pro Pro Ala Ala His His His His His His.

The HER500* construct (SEQ ID NO: 3) was produced by expression of a coding sequence which included, in the 5' to 3' direction: the coding sequence for: a 32 amino acid PAP signal sequence, a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, 3 amino acids of HER-2 signal sequence, 289 amino acids of the mature HER-2 membrane distal extracellular domain, a Ala linker, the OVA-derived immunodominant octapeptide SIINFEKL (OVA₂₅₇₋₂₆₄), 217 amino acids of the HER2 membrane distal intracellular domain and a C-terminal tag consisting of Ala Ala Ala His His His His His His His.

The HER500*rGM-CSF construct (SEQ ID NO: 4) was produced by expression of a coding sequence which included, in the 5' to 3' direction: a 32 amino acid PAP signal sequence, a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, 3 amino acids of HER-2 signal sequence, 289 amino acids of the mature HER-2 membrane distal extracellular domain, an Ala linker, the OVA-derived immunodominant octapeptide SIINFEKL (OVA₂₅₇₋₂₆₄), 217 amino acids of the HER2 membrane distal intracellular domain, an Ala Ala linker, a 127 amino acid mature rat GM-CSF sequence and a C-terminal tag consisting of Gly Ala Pro Pro Pro Pro Pro Ala His His His His His His.

The HER300*rGM-CSF construct (SEQ ID NO: 5) was produced by expression of a coding sequence which included, in the 5' to 3' direction: a 32 amino acid PAP signal sequence, a 3 amino acid sequence of the mature PAP protein, an Ala Arg linker, 3 amino acids of HER-2 signal sequence, 289 amino acids of the mature HER-2 membrane distal extracellular domain, an Ala linker, the OVA-derived immunodominant octapeptide SIINFEKL (OVA₂₅₇₋₂₆₄), an Ala linker, a 127 amino acid mature rat GM-CSF sequence and a C-terminal tag consisting of Gly Ala Pro Pro Pro Pro Pro Ala His His His His His His.

Eight-week old randomized female C57BL/6 mice were given 3 IP injections of 2.5×10^5 Ag-pulsed activated DC in 0.1 ml PBS at 2-weeks intervals. Enriched preparations of activated DC were obtained by incubating female C57BL/6 spleen cells in tissue culture flasks for 2h at 37°C, removing non-adherent cells, and subsequently culturing the remaining adherent cells for 2 days in the presence of 1 μ M ionomycin (Sigma, St. Louis, MO; Czerniecki *et al.*, 1997, *J. Immunol.* 159: 3823; Ridge *et al.*, 1998, *Nature* 393: 474). The DC obtained in this manner were pulsed by 16h co-culture with each of the indicated HER-2 fusion proteins at 1 μ M, washed two times and injected into mice. Two weeks after the last *in vivo* immunization, mice were challenged with an IP injection of 5×10^5 E.HER-2 cells in 0.1 ml PBS. Mice were monitored daily and their survival recorded. The results of two independent experiments are shown in Figs. 2A and 2B, respectively.

While immunization with HER500*rGM-CSF-pulsed DC prevented the tumor growth, treatment with HER300*rGM-CSF had no effect (Fig. 2A). These results are consistent with the results obtained *in vitro* in that they confirm the importance of the intracellular HER-2 domain derived segment in attaining a strong level of Ag presentation in order to generate an effective anti-tumor response. Experimental results shown in Fig. 2B demonstrate that a significant level of *in vivo* protection against a HER-2 expressing tumor can

also be generated (a) when HER500-containing immunogens are either fused to the human GM-CSF (HER500•hGM-CSF), (b) in the absence of any GM-CSF when both an intracellular and extracellular portion of the HER-2 antigen is resnt in the construct (HER500 and HER500*), and (c) in the absence of the OVA-derived peptide SIINFELK (HER500 and HER500•hGM-CSF).

Example 3

In vivo suppression of an established tumor by immunization with Ag-pulsed activated DC

The efficacy of immunotherapy with different HER-2 fusion proteins was further evaluated by administration of the HER-2 fusion proteins to tumor-bearing laboratory mice (*i.e.*, animals that were pre-injected with HER-2 expressing tumor cells). Twelve-week old IP injection of 5×10^5 E.HER-2 cells in 0.1 ml PBS on day 0, followed by 2 IP injections of 2.5×10^5 Ag-pulsed activated DC in 0.1 ml PBS (prepared as described in Example 2) one and 12 days later, respectively. Mice were monitored daily and their survival recorded. As shown in Fig. 3, treatment with DC pulsed with the HER500-containing antigenic constructs (HER500•rGM-CSF, HER500, and HER500•hGM-CSF), exhibited a notable therapeutic effect, considerably prolonging the survival of tumor-bearing mice.

| Description | SEQ ID NO |
|--|-----------|
| HER500 amino acid sequence: MRAAPLLLARAASLSLGLFLLFFWLDRSVLAKELARGAASTQVCTGTMKLRLPASPETHLDMRLHLYQG CQVVQGNLELYLPTNASLSFLQDIQEVQGYVLIHNNQVRQVPLQRLRIVRGTLQFEDNYALAVLDNGDPLN NTTPVTGASPGGLRELQRLSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPM CKGSRCWGESSEDCQSLTRTVCAAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPA LVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSAGGMVHHRHRSSTRSGGGDLTLGLEPSEEE APRSLAPSEGAGSDVFDGDLGMGAAGLQSLPTHDPSPQLQRYSEDPTVPLPSETDGYVAPLTCSPQPEYVNO PDVRPQPPSPREGPLPAARPAGATLERAKTILSPGKNGVVKDVFAFGGAVERNPEYLTQGGGAAPQPHPPAFSP AFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPAAAHHHHHHH | 1 |
| HER500•hGM-CSF amino acid sequence: MRAAPLLLARAASLSLGLFLLFFWLDRSVLAKELARGAASTQVCTGTMKLRLPASPETHLDMRLHLYQG CQVVQGNLELYLPTNASLSFLQDIQEVQGYVLIHNNQVRQVPLQRLRIVRGTLQFEDNYALAVLDNGDPLN NTTPVTGASPGGLRELQRLSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPM CKGSRCWGESSEDCQSLTRTVCAAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPA LVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSAGGMVHHRHRSSTRSGGGDLTLGLEPSEEE APRSLAPSEGAGSDVFDGDLGMGAAGLQSLPTHDPSPQLQRYSEDPTVPLPSETDGYVAPLTCSPQPEYVNO PDVRPQPPSPREGPLPAARPAGATLERAKTILSPGKNGVVKDVFAFGGAVERNPEYLTQGGGAAPQPHPPAFSP AFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPAAPARSPSPSTQPEHVNAIQEARLLNLSRDT AAEMNETVEISEMFDLQEPCLQTRLELYKQGLRSLTKLKGPLTMMASHYKQHCPTPETSCATQITFESF KENLKDFLLVIPFDCWEPVQEGAPPPAAHHHHHHH | 2 |
| HER500* amino acid sequence: MRAAPLLLARAASLSLGLFLLFFWLDRSVLAKELARGAASTQVCTGTMKLRLPASPETHLDMRLHLYQG CQVVQGNLELYLPTNASLSFLQDIQEVQGYVLIHNNQVRQVPLQRLRIVRGTLQFEDNYALAVLDNGDPLN NTTPVTGASPGGLRELQRLSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPM CKGSRCWGESSEDCQSLTRTVCAAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPA LVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSASINFEKLGAGGMVHHRHRSSTRSGGGDLTL GLEPSEEEAPRSLAPSEGAGSDVFDGDLGMGAAGLQSLPTHDPSPQLQRYSEDPTVPLPSETDGYVAPLTCSP QPEYVNOQPDVRPQPPSPREGPLPAARPAGATLERAKTILSPGKNGVVKDVFAFGGAVERNPEYLTQGGGAAPQ HPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPAAAHHHHHHH | 3 |

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| <p>HER500*αrGM-CSF amino acid sequence:</p> <p>MRAAPLLARAASLSLGFLLFFWLDRSVLAKELARGAASTQVCTGTMKLRLPASPETHLDMRLHLYQG CQVVQGNLELTYPNASLSFLQDIQEVQGYVLIHNVQVRQVPLQRLRIVRGTLFEDNYALAVLDNGDPLN NTTPVTGASPGGLRELQLRSLTEILKGGVLIQNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPM CKGSRWCWGESSEDCQSLRTRVCAGGCARCKGPLPTDCHEQCAAGCTGPKHSDCLACLFHFNHSGICELHCPA LVTYNNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSASIHFEKLGAGGMVHHRHRSSTSRGGDLTL GLEPSEEEAPRSPLAPSEGAGSDVFDGDLGMGAAGLQSLPTHDPSPQLQRYSEDPTVPLPSETDGYVAPLTCSF QPEYVNPDPVRPQPPSPREGPLPAARPAGATLERAKTILSPGKNGVVKDVFAFGGAVENPEYLTQGGAAPQP HPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPAAPTRSPNPVTRPWKHVDAIKEALS LLNDMRALENEKNEVDIISNEFSIQRPTCVQTRLKLYKQGLRGNLTKLNGALTMIA SHYQTNCPPPTETDCEI EVTTFEDFIKNLKGFLFDIPDCWKPVQKGAPPPPAHHHHHH</p> | 4 |
| <p>HER300*αrGM-CSF amino acid sequence:</p> <p>MRAAPLLARAASLSLGFLLFFWLDRSVLAKELARGAASTQVCTGTMKLRLPASPETHLDMRLHLYQG CQVVQGNLELTYPNASLSFLQDIQEVQGYVLIHNVQVRQVPLQRLRIVRGTLFEDNYALAVLDNGDPLN NTTPVTGASPGGLRELQLRSLTEILKGGVLIQNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPM CKGSRWCWGESSEDCQSLRTRVCAGGCARCKGPLPTDCHEQCAAGCTGPKHSDCLACLFHFNHSGICELHCPA LVTYNNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSASIHFEKLAAPTRSPNPVTRPWKHVDAIKEALS LLNDMRALENEKNEVDIISNEFSIQRPTCVQTRLKLYKQGLRGNLTKLNGALTMIA SHYQTNCPPPTETDCEI EVTTFEDFIKNLKGFLFDIPDCWKPVQKGAPPPPAHHHHHH</p> | 5 |
| <p>HER500 nucleotide coding sequence:</p> <p>ATGAGAGCTGCACCCCTCCTCTGGCCAGGGCAGCAAGCCTTAGCCTTGCTTCTGTTTCTGCTTTTTT CTGGCTAGACCGAAGTGTACTAGCCAAGGAGTTGGCGCGCGGGGCGCGTGCACCCAAGTGTGCACCGG CACAGACATGAAGCTGCGGCTCCCTGCCAGTCCCGAGACCCACCTGGACATGCTCCGCCACCTTACCA GGGCTGCCAGGTGGTGCAGGGAAACCTGGAACACCTACCTGCCCACCAATGCCAGCCTGTCCTTCTCTG CAGGATATCCAGGAGGTGCAGGGCTACGTGCTCATCGCTCACAACCAAGTGAGGCAGGTCCCACTGCAG AGGCTGCGGATTGTGCGAGGCACCCAGCTCTTTGAGGACAACATATGCCCTGGCCGTGCTAGACAATGGA GACCCGTGAACAATACCACCCCTGTACAGGGGCTCCCAAGGAGGCTGCGGGAGCTGCAGCTCGA AGCCTCACAGAGATCTTGAAGGAGGGGTCTTGATCCAGCGGAACCCCAAGCTCTGCTACCAGGACACG ATTTTGTGGAAGGACATCTTCCACAAGAACAACAGCTGGCTCTCACACTGATAGACACCAACCGCTCTC GGGCTGCCACCCCTGTTCTCCGATGTGTAAGGGCTCCCGCTGCTGGGGAGAGAGTTCTGAGGATTGTCA GAGCCTGACGCGCACTGTCTGTGCGGTTGGCTGTGCCCGCTGCAAGGGGCCACTGCCCACTGACTGCTGC CATGAGCAGTGTGTGCGCGGTGCACGGGCCCCAAGCACTCTGACTGCCTGGCCTGCCTCCACTTCAACC ACAGTGGCATCTGTGAGCTGCACTGCCCAGCCCTGGTCACCTACAACACAGACACGTTTGAGTCCATGCC CAATCCCGAGGGCGGTATACATTCGGCGCCAGCTGTGTGACTGCCTGTCCCTACAACTACCTTCTACG GACGTGGGATCGGGCGTGGGGCATGGTCCACACAGGCACCGCAGCTCATCTACCAGGAGTGGCGGT GGGGACCTGACACTAGGGCTGGAGCCCTCTGAAGAGGAGGCCCCAGGTCTCCACTGGCACCCCTCCGAA GGGGCTGGCTCCGATGATTTGATGGTGACCTGGGAATGGGGGAGCCAAAGGGGCTGCAAAGCCTCCCC ACACATGACCCAGCCCTCTACAGCGGTACAGTGAGGACCCACAGTACCCCTGCCCTCTGAGACTGAT GGCTACGTGCCCCCTGACCTGCAGCCCCAGCCTGAATATGTGAACAGCCAGATGTTTCGGCCCCAGC CCCCCTCGCCCCGAGAGGGCCCTCTGCCTGCTGCCCGACCTGCTGGTGCCACTCTGGAAAGGGCAAGAC TCTCTCCCCAGGGAAGAATGGGGTCTGCAAGACGTTTTTGCCTTTGGGGTGCCGTGGAGAACCCCGAG TACTTGACACCCAGGGAGGAGCTGCCCTCAGCCCCACCTCCTCCTGCTTCAGCCAGCCTTCGACA ACCTCTATTACTGGGACAGGACCCACAGAGCGGGGGCTCCACCCAGCACCTTCAAAGGGACACCTA CGGCAGAGAACCCAGGTACCTGGGTCTGGACGTGCCAGCGCCGCACATCACCATCACCATCAC</p> | 6 |

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| <p>HER500•hGM-CSF nucleotide coding sequence:</p> <p>ATGAGAGCTGCACCCCTCCTCTGGCCAGGGCAGCAAGCCTTAGCCTTGGCTTCTTGTCTGCTTTTTTT CTGGCTAGACCGAAGTGTACTAGCCAAGGAGTTGGCGCGGGGCGCGTGCACCAAGTGTGCACCGG CACAGACATGAAGCTGCGGCTCCCTGCCAGTCCCGAGACCCACCTGGACATGCTCCGCCACCTCTACCA GGGCTGCCAGGTGGTGCAGGGAAACCTGGAACCTACCTACCTGCCACCAATGCCAGCCTGTCTTCTCTG CAGGATATCCAGGAGGTGCAGGGCTACGTGCTCATCGCTCACAACCAAGTGAGGCAGGTCCCACTGCAG AGGCTGCGGATTTGTGCGAGGCACCCAGCTCTTTGAGGACAACCTATGCCCTGGCCGTGCTAGACAATGGA GACCCGCTGAACAATACCACCCCTGTACAGGGGCTCCCCAGGAGGCCTGCGGGAGCTGCAGCTTCGA AGCCTCACAGAGATCTTGAAAGGAGGGGTCTTGATCCAGCGGAACCCCACTGTGCTACCAGGACACG ATTTTGTGGAAGGACATCTTCCACAAGAACAACAGCTGGCTCTCACACTGATAGACACCAACCGCTCTC GGGCTGGCTGCCCTGTTCTCCGATGTGTAAAGGGCTCCCGCTGCTGGGGAGAGAGTTCTGAGGATTGTCA GAGCCTGACGCGCACTGTCTGTGCCGGTGGCTGTGCCCGCTGCAAGGGGGCACTGCCCACTGACTGTCTGC CATGAGCAGTGTGCTGCCGGCTGCACGGGCCCCAAGCACTCTGACTGCCTGGCCTGCCTCCACTTCAACC ACAGTGGCATCTGTGAGCTGCACTGCCAGCCCTGGTCACTACAACACAGACACGTTTGTAGTCCATGCC CAATCCCGAGGGCCGGTATACATTGCGCGCCAGCTGTGTGACTGCCTGTCCCTACAACCTACCTTTCTACG GACGTGGGATCGGGCGCTGGGGGATGGTCCACCACAGGCACCGCAGCTCATCTACCAGGAGTGGCGGT GGGGACCTGACACTAGGGCTGGAGCCCTCTGAAGAGGAGGGCCCCAGGTCTCCACTGGCACCCCTCCGAA GGGCTGAGAAACCCAGAGTATTTGATGGTGACCTGGGAATGGGGGAGAGAGTTCTGAGGATTGTCA ACACATGACCCAGCCCTCTACAGCGGTACAGTGAGGACCCACAGTACCCTGCCCTCTGAGACTGAT GGCTACGTTGCCCCCTGACCTGCAGCCCCAGCCTGAATATGTGAACAGCCAGATGTTGCGCCCCAGC CCCCCTGCCCCGAGAGGGCCCTCTGCCTGCTGCCGACCTGCTGGTGCCACTCTGGAAGGGCCAAGAC TCTCTCCCCAGGGAAGAAATGGGGTCTGTAAGACGTTTTTGCCTTTGGGGGTGCCGTGGAGAACCCCGAG TACTTGACACCCAGGGAGGAGCTGCCCTCAGCCCCACCTCCTCCTGCCTTACGCCAGCCTTCGACA ACCTCTATTACTGGGACCAGGACCCACAGAGCGGGGGCTCCACCCAGCACCTTCAAAGGGACACCTA CGGACAGAGAACCAGAGTACCTGGGTCTGGACGTGCCAGCGGCCGACCCGCCCGCTCGCCAGCCCCA GCACACAGCCCTGGGAGCATGTGAATGCCATCCAGGAGGCCCGGCGTCTCCTGAACCTGAGTAGAGACA CTGCTGCTGAGATGAATGAAACAGTAGAAGTCATCTCAGAAATGTTTGACCTCCAGGAGCCGACCTGCCT ACAGACCCGCCTGGAGCTGTACAAGCAGGGCCTGCGGGGACGCTCACCAGCTCAAGGGCCCCCTTGAC CATGATGGCCAGCCACTACAACAGCACTGCCCTCCAACCCCGGAAACTTCTGTGCAACCCAGATTATC ACCTTTGAAAGTTTCAAAGAGAACCTGAAGGACTTTCTGCTTGTATCCCTTTGACTGCTGGGAGCCAG TCCAGGAGGGCGCGCCACCCCGCGGGCGGCACATCACCATCACCATCAC</p> | 7 |
| <p>HER500* nucleotide coding sequence:</p> <p>ATGAGAGCTGCACCCCTCCTCTGGCCAGGGCAGCAAGCCTTAGCCTTGGCTTCTTGTCTGCTTTTTTT CTGGCTAGACCGAAGTGTACTAGCCAAGGAGTTGGCGCGGGGCGCGTGCACCAAGTGTGCACCGG CACAGACATGAAGCTGCGGCTCCCTGCCAGTCCCGAGACCCACCTGGACATGCTCCGCCACCTCTACCA GGGCTGCCAGGTGGTGCAGGGAAACCTGGAACCTACCTACCTGCCACCAATGCCAGCCTGTCTTCTCTG CAGGATATCCAGGAGGTGCAGGGCTACGTGCTCATCGCTCACAACCAAGTGAGGCAGGTCCCACTGCAG AGGCTGCGGATTTGTGCGAGGCACCCAGCTCTTTGAGGACAACCTATGCCCTGGCCGTGCTAGACAATGGA GACCCGCTGAACAATACCACCCCTGTACAGGGGCTCCCCAGGAGGCCTGCGGGAGCTGCAGCTTCGA AGCCTCACAGAGATCTTGAAAGGAGGGGTCTTGATCCAGCGGAACCCCACTGTGCTACCAGGACACG ATTTTGTGGAAGGACATCTTCCACAAGAACAACAGCTGGCTCTCACACTGATAGACACCAACCGCTCTC GGGCTGCCACCCCTGTTCTCCGATGTGTAAAGGGCTCCCGCTGCTGGGGAGAGAGTTCTGAGGATTGTCA GAGCCTGACGCGCACTGTCTGTGCCGGTGGCTGTGCCCGCTGCAAGGGGGCACTGCCCACTGACTGTCTGC CATGAGCAGTGTGCTGCCGGCTGCACGGGCCCCAAGCACTCTGACTGCCTGGCCTGCCTCCACTTCAACC ACAGTGGCATCTGTGAGCTGCACTGCCAGCCCTGGTCACTACAACACAGACACGTTTGTAGTCCATGCC CAATCCCGAGGGCCGGTATACATTGCGCGCCAGCTGTGTGACTGCCTGTCCCTACAACCTACCTTTCTACG GACGTGGGATCCGCTAGCATCAATTAATTTGAGAAAGTTGGCGCTGGGGGATGGTCCACCACAGGCAC CGCAGCTCATCTACCAGGAGTGGCGGTGGGGACCTGACACTAGGGCTGGAGCCCTCTGAAGAGGAGGCC CCAGGTCTCCACTGGCACCCCTCCGAAGGGGCTGGCTCCGATGTATTTGATGGTGACCTGGGAATGGGGG CAGCCAAGGGGCTGCAAAGCCTCCCCACATGACCCAGCCCTCTACAGCGGTACAGTGAGGACCCCA CAGTACCCCTGCCCTCTGAGACTGATGGCTACGTTGCCCCCTGACCTGCAGCCCCAGCCTGAATATGT GAACCAAGGAGATGTTTCGGCCCCAGCCCCCTTCGCCCCGAGAGGGCCCTCTGCCTGCTGCCGACCTGTCT GGTGCCACTCTGGAAGGGCCAAGACTCTCTCCCGAGGGAAGAATGGGGTCTGTAAGACGTTTTTGGC TTTGGGGGTGCCGTGGAGAACCCGAGTACTTGACACCCAGGGAGGAGTGCCTCAGCCCCACCT CCTCCTGCCTTACGCCAGCCTTCGACAACCTCTATTACTGGGACCAGGACCCACAGAGCGGGGGGCTC CAGCCAGCACCTTCAAAGGGACACCTACGGCAGAGAACCAGAGTACCTGGGTCTGGACGTGCCAGCGG CCGCACATCACCATCACCATCAC</p> | 8 |

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| <p>HER500*<i>r</i>GM-CSF nucleotide coding sequence:</p> <p>ATGAGAGCTGCACCCCTCCTCCTGGCCAGGGCAGCAAGCCTTAGCCTTGGCTTCTTGTITCTGCTTTT TTTCTGGCTAGACCGAAGTGTACTAGCCAAGGAGTTGGCGCGGGGCGCGTCGACCCAAGTGTGC ACCGGCACAGACATGAAGCTGCGGCTCCCTGCCAGTCCCGAGACCCACCTGGACATGCTCCGCCACC TCTACCAGGGCTGCCAGGTGGTGCAGGGAAACCTGGAACCTACCTACCTGCCACCAATGCCAGCCT GTCTTCTCTGCAGGATATCCAGAGGTGCAGGGCTACGTGCTCATCGCTCACAACCAAGTGAGGCAG GTCCCACTGCAGAGGTGCGGATTGTGCGAGGCACCCAGCTCTTTGAGGACAATATGCCCTGGCCG TGCTAGACAATGGAGACCCGCTGAACAATACCAACCCCTGTCACAGGGGCTCCCAAGGAGGCCTGCG GGAGCTGCAGCTTCGAAGCCTCAGAGATCTTGAAAGGAGGGGTCTTGATCCAGCGGAACCCCCAG CTCTGCTACCAGGACACGATTTTGTGGAAGGACATCTCCACAAGAACAACCAAGCTGGCTCTCACAC TGATAGACACCAACCGCTCTCGGGCTGCCACCCCTGTTCTCCGATGTGTAAGGGCTCCCGCTGCTGG GGAGAGAGTTCTGAGGATTGTGAGAGCTGACGCGCACTGTCTGTGCCGGTGGCTGTGCCCGCTGCA AGGGGCCACTGCCCACTGACTGCTGCCATGAGCAGTGTGCTGCCGGCTGCACGGGCCCAAGCACTC TGACTGCTTGGCTGCTCCACTTCAACCAAGTGATGCTGTGAGCTGCACTGCCAGCCCTGGTCA CCTACAACACAGACAGTTTGTAGTCCATGCCAATCCCGAGGGCCGGTATACATTCGGCGCCAGCTG TGTGACTGCCTGTCCCTACAACCTCTTCTACGAGCTGGGATCCGCTAGCATCATTAAATTCGAGA AGTTGGGCGCTGGGGGCATGGTCCACCACAGGCACCGCAGCTCATCTACCAGGAGTGGCGGTGGGA CCTGACACTAGGGCTGGAGCCCTCTGAAGAGGAGGGCCCCAGGTCTCCACTGGCACCCCTCCGAAGGG GCTGGCTCCGATGTATTGATGGTGACCTGGGAATGGGGGCAGCCAAGGGGCTGCAAGCCTCCCA CACATGACCCAGCCCTCTACAGCGGTACAGTGAGGACCCCAAGTACCCTGCCCTCTGAGACTGA TGGCTACGTTGCCCCCTGACCTGCAGCCCCAGCCTGAATATGTGAACAGCCAGATGTTCCGGCCC CAGCCCCCTTCGCCCCGAGAGGGCCCTCTGCCTGCTGCCCGACCTGCTGGTGGCACTCTGGAAGGG CCAAGACTCTCTCCCAAGGAAGAATGGGGTCTGTCAAAGACGTTTTTGCCTTTGGGGGTGCCGTGGA GAACCCCGAGTACTTGACACCCAGGGAGGAGCTGCCCTCAGCCCCACCTCTCTCTGCCTTCAGC CCAGCCTTCGACAACCTCTATTACTGGGACCAGGACCCACAGAGCGGGGGGCTCCACCCAGCACCT TCAAAGGGACACCTACGGCAGAGAACCAGAGTACCTGGGTCTGGAGCTGCCAGCGGGCGCCCCCA CCCGCTCACCAACCCCTGTACCCGGCCCTGGAAGCATGTAGATGCCATCAAAGAAGCTCTGAGCCT CCTAAATGACATGCGTGTCTGGAAGAAGCAAGACGAGTACATCTCTAATGAGTTC TCCATCCAGAGGCCGACATGTGTGCAGACCCGCTGAAGCTATACAAGCAGGTCTACGGGGCAACC TCACCAAACTCAATGGCGCCTTGACCATGATAGCCAGCCACTACCAGACGAACTGCCCTCCAACCCC GGAAACTGACTGTGAAATAGAAGTCACCACCTTTGAGGATTTCAATAAGAACCTTAAAGGCTTTCTG TTTGATATCCCTTTTACTGTGGAAGCCGGTCCAGAAAGGCGCGCCACCCCGCGGCGCATCAC ATCACCATCAC</p> | 9 |
| <p>HER300*<i>r</i>GM-CSF nucleotide coding sequence:</p> <p>ATGAGAGCTGCACCCCTCCTCCTGGCCAGGGCAGCAAGCCTTAGCCTTGGCTTCTTGTITCTGCTTTT TTTCTGGCTAGACCGAAGTGTACTAGCCAAGGAGTTGGCGCGGGGCGCGTCGACCCAAGTGTGC ACCGGCACAGACATGAAGCTGCGGCTCCCTGCCAGTCCCGAGACCCACCTGGACATGCTCCGCCACC TCTACCAGGGCTGCCAGGTGGTGCAGGGAAACCTGGAACCTACCTACCTGCCACCAATGCCAGCCT GTCCTTCTCTGCAGGATATCCAGGAGGTGCAGGGCTACGTGCTCATCGCTCACAACCAAGTGAGGCAG GTCCCACTGCAGAGGTGCGGATTGTGCGAGGCACCCAGCTCTTTGAGGACAATATGCCCTGGCCG TGCTAGACAATGGAGACCCGCTGAACAATACCAACCCCTGTCACAGGGGCTCCCAAGGAGGCCTGCG GGAGCTGCAGCTTCGAAGCCTCAGAGATCTTGAAAGGAGGGGTCTTGATCCAGCGGAACCCCCAG CTCTGCTACCAGGACACGATTTTGTGGAAGGACATCTCCACAAGAACAACCAAGCTGGCTCTCACAC TGATAGACACCAACCGCTCTCGGGCTGCCACCCCTGTTCTCCGATGTGTAAGGGTCCCGCTGCTGG GGAGAGAGTTCTGAGGATTGTGAGAGCTGACGCGCACTGTCTGTGCCGGTGGCTGTGCCCGCTGCA AGGGGCCACTGCCCACTGACTGCTGCCATGAGCAGTGTGCTGCCGGCTGCACGGGCCCAAGCACTC TGACTGCCTGGCCTGCCTCCACTTCAACCACAGTGGCATCTGTGAGCTGCACTGCCAGCCCTGGTCA CCTACAACACAGACAGTTTGTAGTCCATGCCAATCCCGAGGGCCGGTATACATTGCGCGCCAGCTG TGTGACTGCCTGTCCCTACAACCTACCTTTCTACGAGCTGGGATCCGCTAGCATCATTAAATTCGAGA AGTTGGCGGGCCCCACCCGCTCACCAACCCCTGTACCCGGCCCTGGAAGCATGTAGATGCCATCAA AGAAGCTCTGAGCCTCTAAATGACATGCGTGTCTGGAAGAAGCAAGACGAGTACAT CATCTCTAATGAGTTCTCATCCAGAGGCCGACATGTGTGCAGACCCGCTGAAGCTATACAAGCAG GGTCTACGGGGCAACCTACCAAACTCAATGGCGCCTTGACCATGATAGCCAGCCACTACCAGACGA ACTGCCCTCCAACCCCGAAACTGACTGTGAAATAGAAGTCACCACCTTTGAGGATTTCAATAAGAA CCTTAAAGGCTTTCTGTTTGATATCCCTTTTACTGTGGAAGCCGGTCCAGAAAGGCGCGCCACCCC CGCCGGCGCATCACCATCACCATCAC</p> | 10 |

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| 32 amino acid PAP signal sequence: (corresponding to amino acids 1 to 32 of GenBank Accession No. NM_001099) MRAAPLLLARAASLSLGFLFLFFWLDRSVLA | 11 |
| 3 amino acid mature PAP amino acid: (corresponding to amino acids 33 to 35 of GenBank Accession No. NM_001099) KEL | 12 |
| 3 amino acid HER-2 signal sequence: (corresponding to amino acids 19 to 21 GenBank Accession No. M11730) GAA | 13 |
| 2 amino acid linker sequence between mature PAP and HER-2 signal sequence: Ala Arg | 14 |
| C-terminal 9 amino acid sequence found on HER500 and HER500* constructs: Ala Ala Ala His His His His His His | 15 |
| C-terminal 15 amino acid sequence found in HER500-hGM-CSF: Gly Ala Pro Pro Pro Pro Ala Ala His His His His His His | 16 |
| C-terminal 13 amino acid sequence found in HER500* and HER300* rat GM-CSF constructs: Gly Ala Pro Pro Pro Pro Ala His His His His His His | 17 |
| Mature human GM-CSF amino acid sequence: (corresponding to amino acids 18 to 144 GenBank Accession No. NM_000758) APARSPSPSTQPWEHVNAIQEARRLLNLSRDTAAEMNETVEISEMFDLQEPTCLQTRLELYKQGLRGSALT KLKGPLTMMASHYKQHCPTPETSCATQITFESFKENLKDPLLVPFDCWEPVQE | 18 |
| Mature human GM-CSF nucleotide sequence: (corresponding to nucleotides 60 to 440 GenBank Accession No. NM_000758) GCACCCGCCCGCTCGCCAGCCCCAGCACGCGCCCTGGGAGCATGTGAATGCCATCCAGGAGGCCCC GGCGTCTCCTGAACCTGAGTAGAGACACTGCTGCTGAGATGAATGAAACAGTAGAAGTCATCTCAGA AATGTTTGACCTCCAGGAGCCGACCTGCCTACAGACCCGCTGGAGCTGTACAAGCAGGGCCTGCGG GGCAGCCTCACCAAGCTCAAGGGCCCCCTTGACCATGATGGCCAGCCACTACAAGCAGCACTGCCCTC CAACCCCGGAACTTCTGTGCAACCCAGACTATACCTTTGAAAGTTTCAAAGAGAACCTGAAGGA CTTCTGCTTGTCATCCCCCTTGACTGCTGGGAGCCAGTCCAGGAG | 19 |
| Mature rat GM-CSF amino acid sequence: (corresponding to amino acids 1 to 127 GenBank Accession No. U00620) APTRSPNPVTRPWKHVDAIKEALSLLNDMRALENEKNEDVDIISNEFSIQRPCTCVQTRLKLYKQGLRGNLT KLNGALTMIAHYQTNCPTPETDCEIEVTTTFEDFIKNLKGFLFDIPFDCWKPVQK | 20 |
| Mature rat GM-CSF nucleotide sequence: (corresponding to nucleotide 1 to 381 GenBank Accession No. U00620) GCACCCACCCGCTCACCAACCCTGTACCCGGCCCTGGAAGCATGTAGATGCCATCAAAGAAGCTC TGAGCCTCCTAAATGACATGCGTGCTCTGGAGAACGAAAGAAGCAGTAGACATCATCTCTAA TGAGTTCTCCATCCAGAGGCCGACATGTGTGCAGACCCGCTGAAGCTATACAAGCAGGGTCTACGG GGCAACCTCACCAAACTCAATGGCGCCTTGACCATGATAGCCAGCCACTACCAGACGAAGTGCCTC CAACCCCGGAACTGACTGTGAAATAGAAGTCACCACCTTTGAGGATTTTCAAAGAACCCTTAAAGG CTTCTGTTTGATATCCCTTTTGACTGCTGGAAGCCGGTCCAGAAA | 21 |
| Reporter peptide in constructs HER500* and HER500*ratGM-CSF: (OVA-derived immunodominant octapeptide SIINFEKL (OVA ₂₅₇₋₂₆₄)) | 22 |
| 289 amino acids of mature HER-2 membrane distal extracellular domain: (amino acids 22 to 310 of GenBank Accession No. M11730) STQVCTGTDMLRLPASPETHLDMLRHLYQGCQVVGQNLLETYLPNASLSFLQDIQEVQGYVLIHNPQ VRQVPLQLRLVRGTQLFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQL CYQDTILWKDIFHKNNQLALTIDTNRSRACHPCSPMCKGSRGWGESSEDCQSLTRTVCAAGGCARCKGPL PTDCCHQCAAGCTGPKHSDCLACLFHNSHICELHCPALVTYNTDTFESMPNPEGRTYTFGASCVTACPY NYLSTDVGS | 23 |

| | |
|--|----|
| coding sequence for 289 amino acids of mature HER-2 membrane distal extracellular domain: (nucleotides 214 to 1080 of GenBank Accession No. M11730) AGCACCCAAGTGTGACCGGCACAGACATGAAGCTGCGGCTCCCTGCCAGTCCCGAGACCCACCTGG ACATGCTCCGCCACCTCTACCAGGGCTGCCAGGTGGTGACAGGAAACCTGGAACTACCTACCTGCGC CACC AATGCCAGCCTGTCTTCTGTCAGGATATCCAGGAGGTGCAGGGCTACGTGCTCATCGCTCAC AACCAAGTGAGGCAGGTCCCACTGCAGAGGCTGCGGATTGTGCGAGGCACCCAGCTCTTTGAGGACA ACTATGCCCTGGCCGTGCTAGACAATGGAGACCCGCTGAACAATACCACCCCTGTACAGGGGCCTC CCCAGGAGGCCTGCGGGAGCTGCAGCTTCGAAGCCTCACAGAGATCTTGAAAGGAGGGGTCTTGATC CAGCGGAACCCCACTCTGCTACCAGGACACGATTTTGTGGAAGGACATCTTCCACAAGAAACAACC AGCTGGCTCTCACACTGATAGACACCAACCGCTCTCGGGCCTGCCACCCCTGTTCTCCGATGTGTAAG GGCTCCCGCTGCTGCGGAGAGATTCTGAGGATTGTGACAGCCTGACGCGCACTGTCTGTGCGGTG GCTGTGCGCGCTGCAAGGGGCCACTGCCACTGACTGCTGCCATGAGCAGTGTGCTGCCGGCTGCAC GGGCCCCAAGCACTCTGACTGCCTGGCCTGCCTCCACTTCAACCACAGTGGCATCTGTGAGCTGCAC TGCCAGCCCTGGTCACTACAACACAGACACGTTTGTAGTCCATGCCCAATCCCGAGGGCCGGTATA CATTCGGCGCCAGTGTGTGACTGCCTGTCCCTACAACCTACCTTTCTACGGACGTGGGATCC | 24 |
| 217 amino acids of the membrane distal intracellular HER-2 domain: (amino acids 1038 to 1254 of GenBank Accession No. M11730) GAGGMVHHRHRSSTRSGGDLTLGLEPSEEEAPRSPLAPSEAGSDVFDGLGMGAAGLQSLPTHDP PLQRYSEDPTVPLPSETDGYVAPLTCSPQPEYVNPDPVRPQPPSPREGPLPAARPAGATLERAKT LSPGKN GVVKDVFAGGAVENPEYLT PQGGAAPQPHPPAFSPAFDNL YYWDQDPPERGAPPSTFKGTPTAENPEY LGLDVP | 25 |
| coding sequence for 217 amino acids of the membrane distal intracellular HER-2 domain: (nucleotides 3262 to 3912 of GenBank Accession No. M11730) GGCGCTGGGGGCATGGTCCACCACAGGCACCGCAGCTCATCTACCAGGAGTGCGGTGGGGACCTGA CACTAGGGCTGGAGCCCTCTGAAGAGGAGGCCCCAGGTCTCCACTGGCACCCCTCCGAAGGGGCTGG CTCCGATGTATTGATGGTGACCTGGGAATGGGGGCAGCCAAGGGGCTGCAAAGCCTCCACACAT GACCCAGCCCTCTACAGCGGTACAGTGAGGACCCACAGTACCCCTGCCCTCTGAGACTGATGGCTA CGTTGCCCCCTGACCTGCAGCCCCAGCCTGAATATGTGAACAGCCAGATGTTGGCCCCAGCCCC CTTCGCCCCGAGAGGGCCCTCTGCCTGCTGCCGACCTGCTGGTGCCACTCTGGAAGGGCCAAGACT CTCTCCCCAGGGAAGAATGGGGTCGTCAAAGACGTTTTCCTTTGGGGGTGCCGTGGAGAACCCCGA GTACTTGACACCCAGGGAGGAGCTGCCCTCAGCCCCACCTCCTCCTGCCTTCAGCCCAGCCTTCG ACAACCTCTATTACTGGGACCAGGACCCACAGAGCGGGGGCTCCACCCAGCACCTTCAAAGGGAC ACCTACGGCAGAGAACCCAGAGTACCTGGGTCTGGACGTGCCA | 26 |
| NY-ESO-IC Amino Acid Sequence: amino acids 1 to 180 of GenBank Accession No. U87459 fused to the 217 amino acids of membrane distal intracellular HER-2 domain (amino acids 1038 to 1254 of GenBank Accession No. M11730) | 27 |
| NY-ESO-IC DNA Sequence: nucleotides 54 to 593 of GenBank Accession No. U87459 fused to the coding sequence for the 217 amino acids of the membrane distal intracellular HER-2 domain (nucleotides 3262 to 3912 of GenBank Accession No. M11730): | 28 |
| SART3-IC Amino Acid Sequence: amino acids 1 to 962 of GenBank Accession No. AB020880 fused to the 217 amino acids of membrane distal intracellular HER-2 domain (amino acids 1038 to 1254 of GenBank Accession No. M11730) | 29 |
| SART3-IC DNA Sequence: nucleotides 20 to 2905 of GenBank Accession No. AB020880 fused to the coding sequence for the 217 amino acids of the membrane distal intracellular HER-2 domain (nucleotides 3262 to 3912 of GenBank Accession No. M11730) | 30 |

IT IS CLAIMED

1. An immunostimulatory fusion protein, comprising
a polypeptide or protein antigen sequence component and a sequence component derived
5 from the intracellular domain of the HER-2 protein where said immunostimulatory fusion protein
is effective to elicit a cellular immune response to the polypeptide or protein antigen sequence
component of the fusion protein.
2. The immunostimulatory fusion protein of claim 1, wherein the HER-2 intracellular
10 domain sequence component has the sequence presented as SEQ. ID. NO: 25.
3. The immunostimulatory fusion protein of claim 1, wherein the polypeptide or protein
component is associated with tumor cells.
- 15 4. The immunostimulatory fusion protein of claim 1, wherein said fusion protein is
produced by translation of a continuous nucleic acid coding sequence.
5. The immunostimulatory fusion protein of claim 1, wherein the fusion protein is
produced by chemical coupling.
20
6. The immunostimulatory fusion protein of claim 1, wherein the polypeptide or protein
component is the mature HER-2 membrane distal extracellular domain sequence presented as
SEQ. ID. NO: 23.
- 25 7. The immunostimulatory HER2 fusion protein of claim 6, where the HER-2 fusion
protein is selected from the group consisting of HER500 (SEQ ID NO: 1), HER500•hGM-CSF
(SEQ ID NO: 2), HER500* (SEQ ID NO:3) and HER500*•rGM-CSF (SEQ ID NO: 4).
8. The immunostimulatory HER2 fusion protein of claim 7, where the fusion protein is
30 HER500•hGM-CSF (SEQ ID NO: 2).
9. The immunostimulatory HER2 fusion protein of claim 1, where the cellular immune
response is a dendritic cell-induced, T cell-mediated immune response.
- 35 10. An immunostimulatory fusion protein composition, comprising
dendritic cells superactivated by *in vitro* exposure to an immunostimulatory fusion protein
of claim 1.
11. An immunostimulatory fusion protein composition according to claim 10, further
40 comprising the immunostimulatory fusion protein of claim 1.

12. A method of producing superactivated DC, by exposure to an immunostimulatory fusion protein, comprising

5 exposing a dendritic cell (DC) or dendritic cell precursor (DCP) to an immunostimulatory fusion protein comprising a polypeptide or protein antigen sequence component and a sequence component derived from the intracellular domain of the HER-2 protein, in a manner effective to result in a cellular immune response to the polypeptide or protein antigen sequence component of the fusion protein.

10 13. The method of claim 12, where said exposing takes place *in vitro*.

14. The method of claim 12, where said exposing takes place *in vivo*.

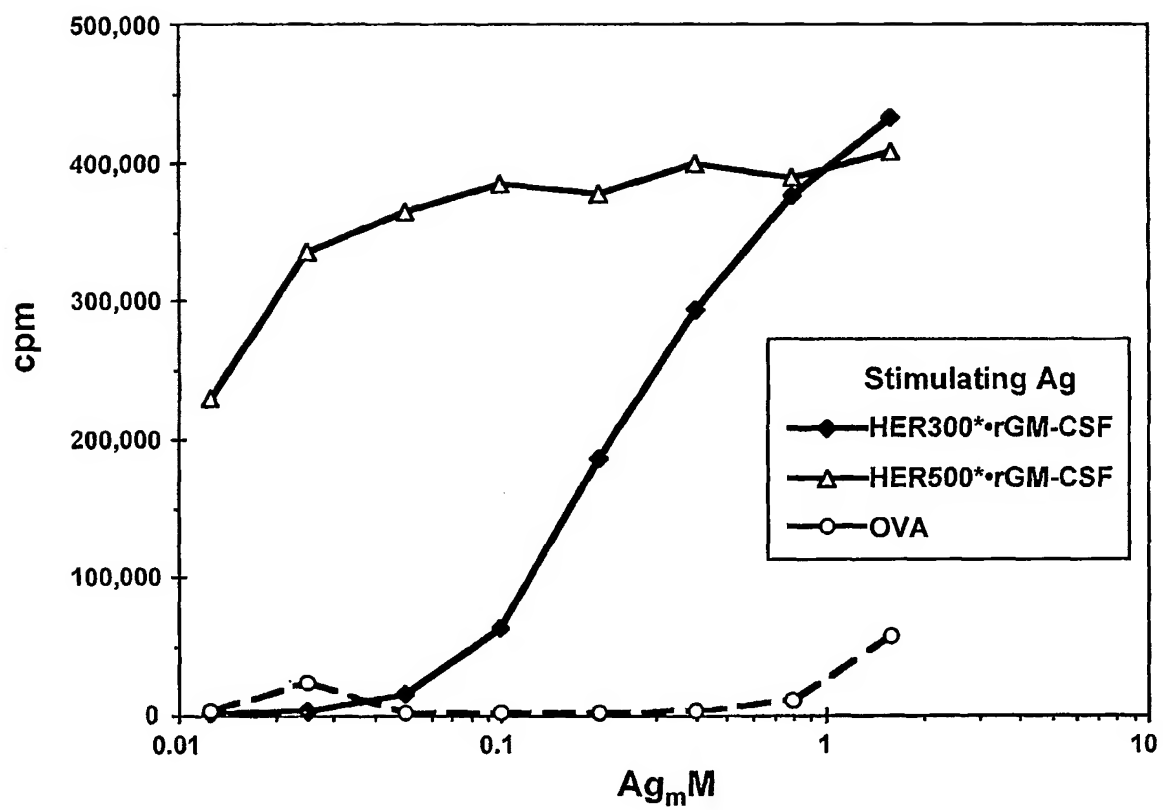
15 15. Use of an immunostimulatory HER2 fusion protein according to any one of claims 1 to 9 in treating cancer where the cancer is associated with a particular antigen.

16. Use of superactivated DC according to any one of claims 12 to 14 in treating cancer where the cancer is associated with a particular antigen.

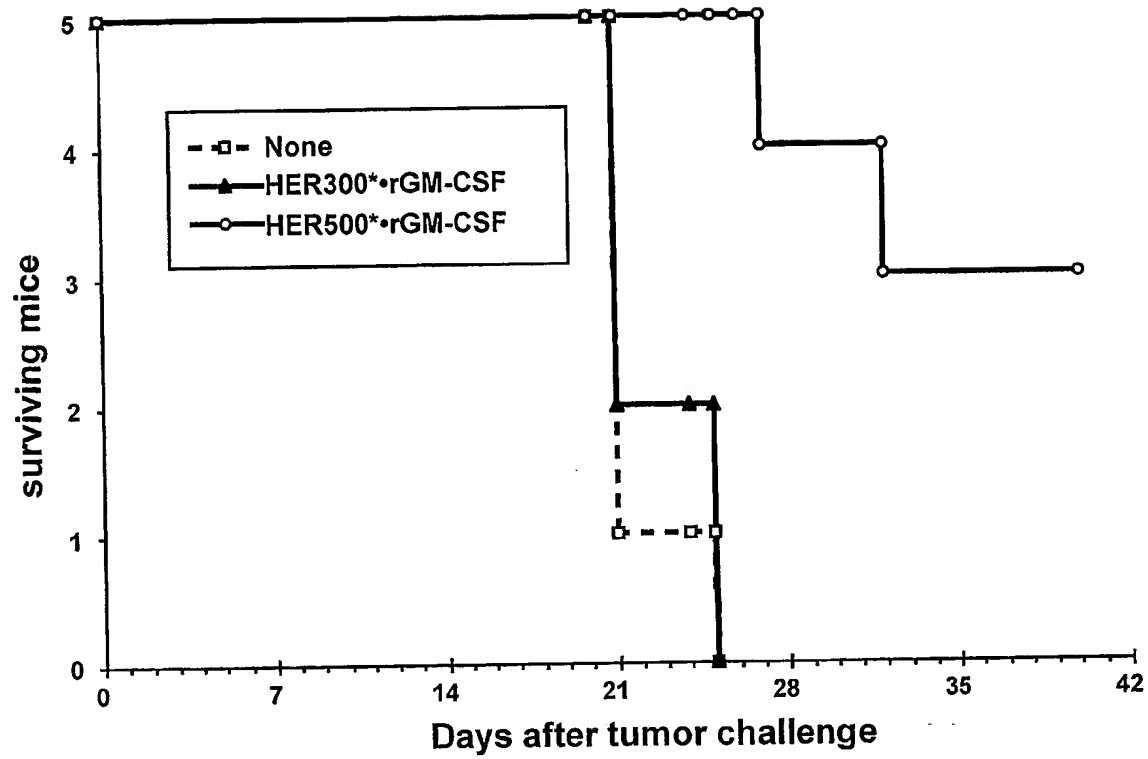
20 17. The use of an immunostimulatory HER2 fusion protein according to any one of claims 1 to 9 in the manufacture of a medicament for treating cancer where the cancer is associated with a particular antigen.

25 18. The use of superactivated DC according to any one of claims 12 to 14 in the manufacture of a medicament for treating cancer where the cancer is associated with a particular antigen.

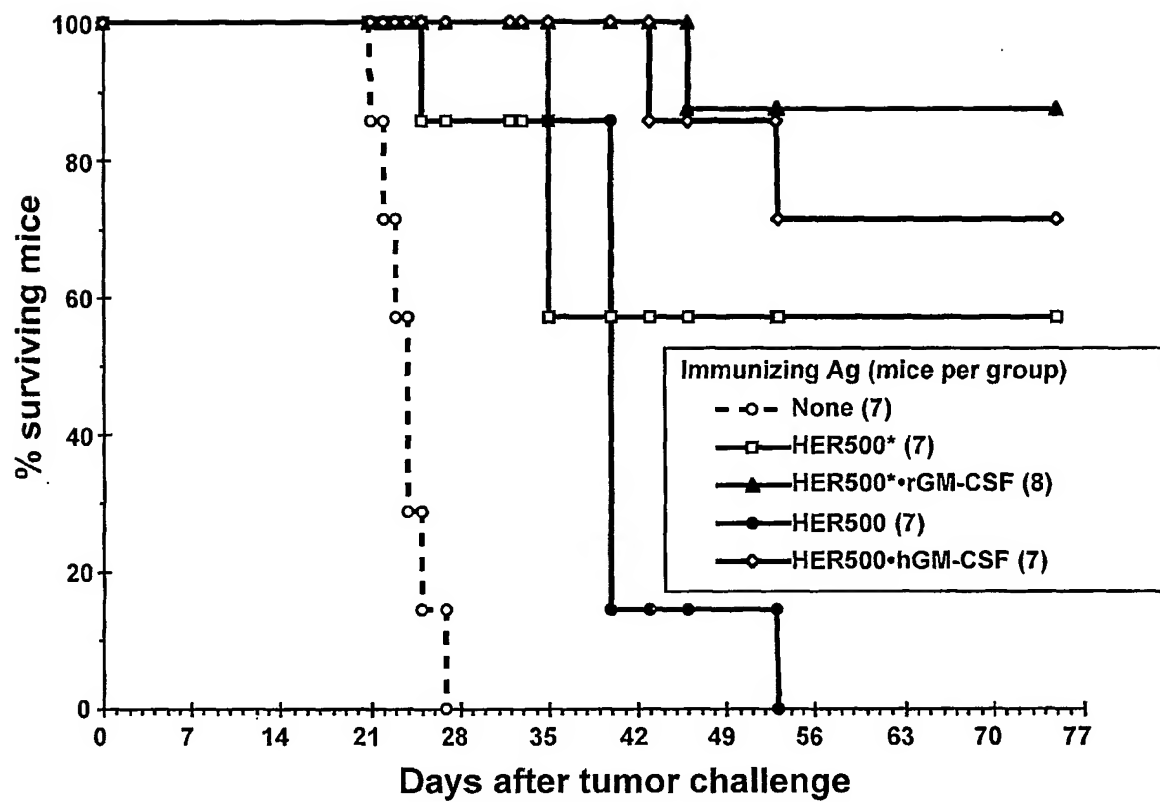
1 / 4

**Fig. 1**

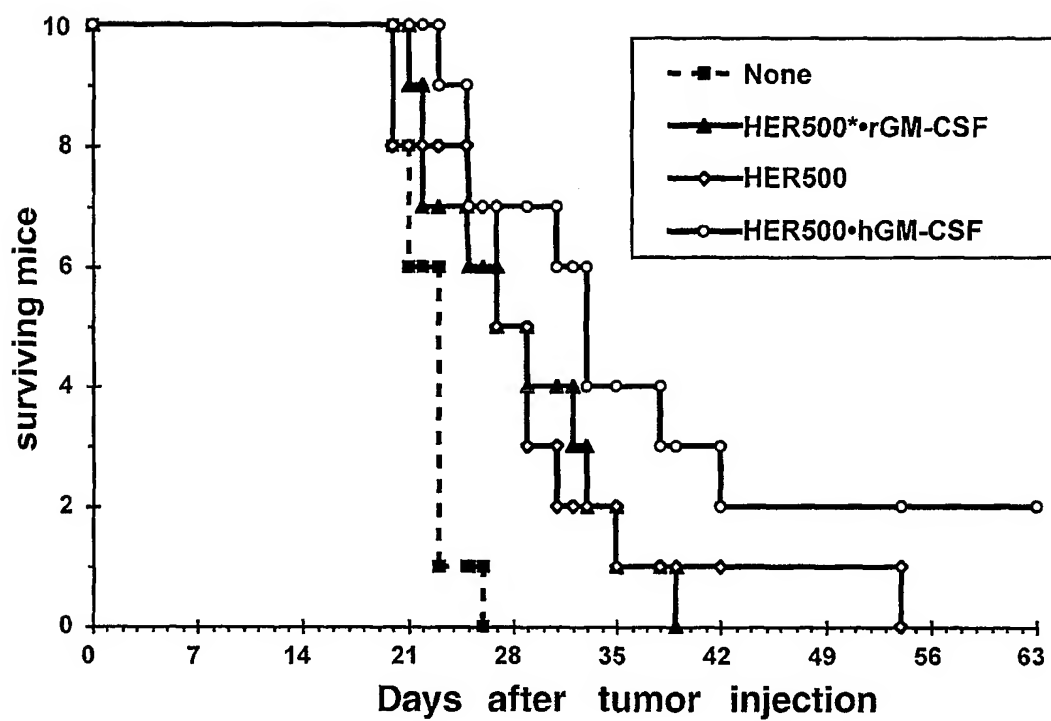
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**Fig. 2A**

3 / 4

**Fig. 2B**

4 / 4

**Fig. 3**

SEQUENCE LISTING

<110> Dendreon Corporation

<120> Compositions and Methods for Dendritic
Cell-Based Immunotherapy

<130> 7636-0022.41

<140> Not Yet Assigned

<141> Filed Herewith

<150> US 60/193,504

<151> 2000-03-30

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<213> Artificial Sequence

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| Gly | Phe | Leu | Phe | Leu | Leu | Phe | Phe | Trp | Leu | Asp | Arg | Ser | Val | Leu | Ala |
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| Lys | Glu | Leu | Ala | Arg | Gly | Ala | Ala | Ser | Thr | Gln | Val | Cys | Thr | Gly | Thr |
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| Leu | Thr | Tyr | Leu | Pro | Thr | Asn | Ala | Ser | Leu | Ser | Phe | Leu | Gln | Asp | Ile |
| | | | 85 | | | | | 90 | | | | | 95 | | |
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| | | 100 | | | | | 105 | | | | | 110 | | | |
| Val | Pro | Leu | Gln | Arg | Leu | Arg | Ile | Val | Arg | Gly | Thr | Gln | Leu | Phe | Glu |
| | 115 | | | | | 120 | | | | | | 125 | | | |
| Asp | Asn | Tyr | Ala | Leu | Ala | Val | Leu | Asp | Asn | Gly | Asp | Pro | Leu | Asn | Asn |
| | 130 | | | | | 135 | | | | 140 | | | | | |
| Thr | Thr | Pro | Val | Thr | Gly | Ala | Ser | Pro | Gly | Gly | Leu | Arg | Glu | Leu | Gln |
| 145 | | | | | 150 | | | | 155 | | | | | 160 | |
| Leu | Arg | Ser | Leu | Thr | Glu | Ile | Leu | Lys | Gly | Gly | Val | Leu | Ile | Gln | Arg |
| | | | 165 | | | | | 170 | | | | | 175 | | |
| Asn | Pro | Gln | Leu | Cys | Tyr | Gln | Asp | Thr | Ile | Leu | Trp | Lys | Asp | Ile | Phe |
| | | 180 | | | | | 185 | | | | | | 190 | | |
| His | Lys | Asn | Asn | Gln | Leu | Ala | Leu | Thr | Leu | Ile | Asp | Thr | Asn | Arg | Ser |
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<213> Artificial Sequence

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| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
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| Pro | Ser | Glu | Gly | Ala | Gly | Ser | Asp | Val | Phe | Asp | Gly | Asp | Leu | Gly | Met |
| | | 370 | | | | 375 | | | | | 380 | | | | |
| Gly | Ala | Ala | Lys | Gly | Leu | Gln | Ser | Leu | Pro | Thr | His | Asp | Pro | Ser | Pro |
| | | | | | 390 | | | | | 395 | | | | | 400 |
| Leu | Gln | Arg | Tyr | Ser | Glu | Asp | Pro | Thr | Val | Pro | Leu | Pro | Ser | Glu | Thr |
| | | | | | 405 | | | | 410 | | | | | 415 | |
| Asp | Gly | Tyr | Val | Ala | Pro | Leu | Thr | Cys | Ser | Pro | Gln | Pro | Glu | Tyr | Val |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Asn | Gln | Pro | Asp | Val | Arg | Pro | Gln | Pro | Pro | Ser | Pro | Arg | Glu | Gly | Pro |
| | | 435 | | | | | 440 | | | | | 445 | | | |
| Leu | Pro | Ala | Ala | Arg | Pro | Ala | Gly | Ala | Thr | Leu | Glu | Arg | Ala | Lys | Thr |
| | | 450 | | | | 455 | | | | | 460 | | | | |
| Leu | Ser | Pro | Gly | Lys | Asn | Gly | Val | Val | Lys | Asp | Val | Phe | Ala | Phe | Gly |
| | | | | | 470 | | | | | 475 | | | | | 480 |
| Gly | Ala | Val | Glu | Asn | Pro | Glu | Tyr | Leu | Thr | Pro | Gln | Gly | Gly | Ala | Ala |
| | | | | | 485 | | | | 490 | | | | | 495 | |
| Pro | Gln | Pro | His | Pro | Pro | Pro | Ala | Phe | Ser | Pro | Ala | Phe | Asp | Asn | Leu |
| | | | 500 | | | | | 505 | | | | | 510 | | |
| Tyr | Tyr | Trp | Asp | Gln | Asp | Pro | Pro | Glu | Arg | Gly | Ala | Pro | Pro | Ser | Thr |
| | | | 515 | | | | 520 | | | | | | 525 | | |
| Phe | Lys | Gly | Thr | Pro | Thr | Ala | Glu | Asn | Pro | Glu | Tyr | Leu | Gly | Leu | Asp |
| | | 530 | | | | 535 | | | | | 540 | | | | |
| Val | Pro | Ala | Ala | Ala | Pro | Ala | Arg | Ser | Pro | Ser | Pro | Ser | Thr | Gln | Pro |
| | | | | | 550 | | | | | 555 | | | | | 560 |
| Trp | Glu | His | Val | Asn | Ala | Ile | Gln | Glu | Ala | Arg | Arg | Leu | Leu | Asn | Leu |
| | | | | | 565 | | | | 570 | | | | | 575 | |
| Ser | Arg | Asp | Thr | Ala | Ala | Glu | Met | Asn | Glu | Thr | Val | Glu | Val | Ile | Ser |
| | | | 580 | | | | | 585 | | | | | 590 | | |
| Glu | Met | Phe | Asp | Leu | Gln | Glu | Pro | Thr | Cys | Leu | Gln | Thr | Arg | Leu | Glu |
| | | 595 | | | | | 600 | | | | | 605 | | | |

Leu Tyr Lys Gln Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro
 610 615 620
 Leu Thr Met Met Ala Ser His Tyr Lys Gln His Cys Pro Pro Thr Pro
 625 630 635 640
 Glu Thr Ser Cys Ala Thr Gln Ile Ile Thr Phe Glu Ser Phe Lys Glu
 645 650 655
 Asn Leu Lys Asp Phe Leu Leu Val Ile Pro Phe Asp Cys Trp Glu Pro
 660 665 670
 Val Gln Glu Gly Ala Pro Pro Pro Pro Ala Ala Ala His His His His
 675 680 685
 His His
 690

<210> 3
 <211> 564
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HER500* construct

<400> 3
 Met Arg Ala Ala Pro Leu Leu Leu Ala Arg Ala Ala Ser Leu Ser Leu
 1 5 10 15
 Gly Phe Leu Phe Leu Leu Phe Phe Trp Leu Asp Arg Ser Val Leu Ala
 20 25 30
 Lys Glu Leu Ala Arg Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr
 35 40 45
 Asp Met Lys Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met
 50 55 60
 Leu Arg His Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu
 65 70 75 80
 Leu Thr Tyr Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile
 85 90 95
 Gln Glu Val Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln
 100 105 110
 Val Pro Leu Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu
 115 120 125
 Asp Asn Tyr Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn
 130 135 140
 Thr Thr Pro Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln
 145 150 155 160
 Leu Arg Ser Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg
 165 170 175
 Asn Pro Gln Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe
 180 185 190
 His Lys Asn Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser
 195 200 205
 Arg Ala Cys His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp
 210 215 220
 Gly Glu Ser Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala
 225 230 235 240
 Gly Gly Cys Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His
 245 250 255
 Glu Gln Cys Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu
 260 265 270
 Ala Cys Leu His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro
 275 280 285
 Ala Leu Val Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro
 290 295 300
 Glu Gly Arg Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr
 305 310 315 320

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Asn Tyr Leu Ser Thr Asp Val Gly Ser Ala Ser Ile Ile Asn Phe Glu
      325      330      335
Lys Leu Gly Ala Gly Gly Met Val His His Arg His Arg Ser Ser Ser
      340      345      350
Thr Arg Ser Gly Gly Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu
      355      360      365
Glu Glu Ala Pro Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser
      370      375      380
Asp Val Phe Asp Gly Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln
      385      390      395      400
Ser Leu Pro Thr His Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp
      405      410      415
Pro Thr Val Pro Leu Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu
      420      425      430
Thr Cys Ser Pro Gln Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro
      435      440      445
Gln Pro Pro Ser Pro Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala
      450      455      460
Gly Ala Thr Leu Glu Arg Ala Lys Thr Leu Ser Pro Gly Lys Asn Gly
      465      470      475      480
Val Val Lys Asp Val Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu
      485      490      495
Tyr Leu Thr Pro Gln Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro
      500      505      510
Ala Phe Ser Pro Ala Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro
      515      520      525
Pro Glu Arg Gly Ala Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala
      530      535      540
Glu Asn Pro Glu Tyr Leu Gly Leu Asp Val Pro Ala Ala Ala His His
      545      550      555      560
His His His His

```

<210> 4

<211> 697

<212> PRT

<213> Artificial Sequence

<220>

<223> HER500*-rGM-CSF construct

<400> 4

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Met Arg Ala Ala Pro Leu Leu Leu Ala Arg Ala Ala Ser Leu Ser Leu
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Gly Phe Leu Phe Leu Leu Phe Phe Trp Leu Asp Arg Ser Val Leu Ala
      20      25      30
Lys Glu Leu Ala Arg Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr
      35      40      45
Asp Met Lys Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met
      50      55      60
Leu Arg His Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu
      65      70      75      80
Leu Thr Tyr Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile
      85      90      95
Gln Glu Val Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln
      100      105      110
Val Pro Leu Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu
      115      120      125
Asp Asn Tyr Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn
      130      135      140
Thr Thr Pro Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln
      145      150      155      160

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Leu Arg Ser Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg
 165 170 175
 Asn Pro Gln Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe
 180 185 190
 His Lys Asn Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser
 195 200 205
 Arg Ala Cys His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp
 210 215 220
 Gly Glu Ser Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala
 225 230 235 240
 Gly Gly Cys Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His
 245 250 255
 Glu Gln Cys Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu
 260 265 270
 Ala Cys Leu His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro
 275 280 285
 Ala Leu Val Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro
 290 295 300
 Glu Gly Arg Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr
 305 310 315 320
 Asn Tyr Leu Ser Thr Asp Val Gly Ser Ala Ser Ile Ile Asn Phe Glu
 325 330 335
 Lys Leu Gly Ala Gly Gly Met Val His His Arg His Arg Ser Ser Ser
 340 345 350
 Thr Arg Ser Gly Gly Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu
 355 360 365
 Glu Glu Ala Pro Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser
 370 375 380
 Asp Val Phe Asp Gly Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln
 385 390 395 400
 Ser Leu Pro Thr His Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp
 405 410 415
 Pro Thr Val Pro Leu Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu
 420 425 430
 Thr Cys Ser Pro Gln Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro
 435 440 445
 Gln Pro Pro Ser Pro Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala
 450 455 460
 Gly Ala Thr Leu Glu Arg Ala Lys Thr Leu Ser Pro Gly Lys Asn Gly
 465 470 475 480
 Val Val Lys Asp Val Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu
 485 490 495
 Tyr Leu Thr Pro Gln Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro
 500 505 510
 Ala Phe Ser Pro Ala Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro
 515 520 525
 Pro Glu Arg Gly Ala Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala
 530 535 540
 Glu Asn Pro Glu Tyr Leu Gly Leu Asp Val Pro Ala Ala Ala Pro Thr
 545 550 555 560
 Arg Ser Pro Asn Pro Val Thr Arg Pro Trp Lys His Val Asp Ala Ile
 565 570 575
 Lys Glu Ala Leu Ser Leu Leu Asn Asp Met Arg Ala Leu Glu Asn Glu
 580 585 590
 Lys Asn Glu Asp Val Asp Ile Ile Ser Asn Glu Phe Ser Ile Gln Arg
 595 600 605
 Pro Thr Cys Val Gln Thr Arg Leu Lys Leu Tyr Lys Gln Gly Leu Arg
 610 615 620
 Gly Asn Leu Thr Lys Leu Asn Gly Ala Leu Thr Met Ile Ala Ser His
 625 630 635 640
 Tyr Gln Thr Asn Cys Pro Pro Thr Pro Glu Thr Asp Cys Glu Ile Glu
 645 650 655

Val Thr Thr Phe Glu Asp Phe Ile Lys Asn Leu Lys Gly Phe Leu Phe
 660 665 670
 Asp Ile Pro Phe Asp Cys Trp Lys Pro Val Gln Lys Gly Ala Pro Pro
 675 680 685
 Pro Pro Ala His His His His His His
 690 695

<210> 5

<211> 479

<212> PRT

<213> Artificial Sequence

<220>

<223> HER300*-rGM-CSF construct

<400> 5

Met Arg Ala Ala Pro Leu Leu Leu Ala Arg Ala Ala Ser Leu Ser Leu
 1 5 10 15
 Gly Phe Leu Phe Leu Leu Phe Phe Trp Leu Asp Arg Ser Val Leu Ala
 20 25 30
 Lys Glu Leu Ala Arg Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr
 35 40 45
 Asp Met Lys Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met
 50 55 60
 Leu Arg His Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu
 65 70 75 80
 Leu Thr Tyr Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile
 85 90 95
 Gln Glu Val Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln
 100 105 110
 Val Pro Leu Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu
 115 120 125
 Asp Asn Tyr Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn
 130 135 140
 Thr Thr Pro Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln
 145 150 155 160
 Leu Arg Ser Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg
 165 170 175
 Asn Pro Gln Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe
 180 185 190
 His Lys Asn Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser
 195 200 205
 Arg Ala Cys His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp
 210 215 220
 Gly Glu Ser Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala
 225 230 235 240
 Gly Gly Cys Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His
 245 250 255
 Glu Gln Cys Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu
 260 265 270
 Ala Cys Leu His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro
 275 280 285
 Ala Leu Val Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro
 290 295 300
 Glu Gly Arg Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr
 305 310 315 320
 Asn Tyr Leu Ser Thr Asp Val Gly Ser Ala Ser Ile Ile Asn Phe Glu
 325 330 335
 Lys Leu Ala Ala Pro Thr Arg Ser Pro Asn Pro Val Thr Arg Pro Trp
 340 345 350
 Lys His Val Asp Ala Ile Lys Glu Ala Leu Ser Leu Leu Asn Asp Met
 355 360 365

Arg Ala Leu Glu Asn Glu Lys Asn Glu Asp Val Asp Ile Ile Ser Asn
 370 375 380
 Glu Phe Ser Ile Gln Arg Pro Thr Cys Val Gln Thr Arg Leu Lys Leu
 385 390 395 400
 Tyr Lys Gln Gly Leu Arg Gly Asn Leu Thr Lys Leu Asn Gly Ala Leu
 405 410 415
 Thr Met Ile Ala Ser His Tyr Gln Thr Asn Cys Pro Pro Thr Pro Glu
 420 425 430
 Thr Asp Cys Glu Ile Glu Val Thr Thr Phe Glu Asp Phe Ile Lys Asn
 435 440 445
 Leu Lys Gly Phe Leu Phe Asp Ile Pro Phe Asp Cys Trp Lys Pro Val
 450 455 460
 Gln Lys Gly Ala Pro Pro Pro Pro Ala His His His His His His
 465 470 475

<210> 6
 <211> 1665
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> HER500 construct

<400> 6
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 ctgctttttt tctggctaga ccgaagtgt ctagccaagg agttggcgcg cggggccgcg 120
 tcgacccaag tgtgcaccgg cacagacatg aagctgcggc tccctgccag tcccgagacc 180
 cacctggaca tgctccgcca cctctaccag ggctgccagg tgggtgcagg aaacctggaa 240
 ctacacctacc tgcccaccaa tgccagcctg tccttctctg aggatattcca ggaggtgcag 300
 ggctacgtgc tcatcgctca caaccaagtg aggcaggctc cactgcagag gctgcggtatt 360
 gtgcgaggca cccagctctt tgaggacaac tatgccctgg ccgtgctaga caatggagac 420
 ccgctgaaca ataccacccc tgtcacaggg gcctcccag gaggcctgcg ggagctgcag 480
 cttcgaagcc tcacagagat cttgaaagga ggggtcttga tccagcggaa ccccagctc 540
 tgctaccagg acacgatttt gtggaaggac atcttccaca agaacaacca gctggctctc 600
 acaactgatag acaccaaccg ctctcgggcg tgccaccct gttctccgat gtgtaagggc 660
 tcccgctgct ggggagagag ttctgaggat tgtcagagcc tgacgcgcac tgtctgtgcc 720
 ggtggctgtg cccgctgcaa ggggccactg cccactgact gctgccatga gcagtgtgct 780
 gccggctgca cgggcccaca gcaactctgac tgccctggcct gcctccactt caaccacagt 840
 ggcatctgtg agctgactg cccagccctg gtcacctaca acacagacac gtttgagtcc 900
 atgcccattc ccgagggccg gtatacattc ggcgccagct gtgtgactgc ctgtccctac 960
 aactaccttt ctacggacgt gggatcgggc gctgggggca tgggtccacca caggcaccgc 1020
 agctcatcta ccaggagtgg cgggtggggac ctgacactag ggctggagcc ctctgaagag 1080
 gaggccccca ggtctccact ggcacctcc gaaggggctg gctccgatgt atttgatggt 1140
 gacctgggaa tgggggcagc caaggggctg caaacctcc ccacacatga cccagccct 1200
 ctacagcggg acagtgagga cccacagta cccctgccct ctgagactga tggctacgtt 1260
 gccccctga cctgcagccc ccagcctgaa tatgtgaacc agccagatgt tcggccccag 1320
 ccccttctgc cccgagaggg cctctgcct gctgcccagc ctgctggtgc cactctggaa 1380
 agggccaaga ctctctcccc agggaagaat ggggtcgtca aagacgtttt tgcctttggg 1440
 ggtgccgtgg agaaccccga gtacttgaca cccagggag gagctgcccc tcagccccac 1500
 cctctctctg ccttcagccc agccttcgac aacctctatt actgggacca ggaccacca 1560
 gagcgggggg ctccaccag caccctcaaa gggcaccta cggcagagaa cccagagtac 1620
 ctgggtctgg acgtgccagc ggccgcacat caccatcacc atcac 1665

<210> 7
 <211> 2070
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> HER500-hGM-CSF construct

<400> 7

| | | | | | | |
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| atgagagctg | cacccctcct | cctggccagg | gcagcaagcc | ttagccttgg | cttcttggtt | 60 |
| ctgctttttt | tctggctaga | ccgaagtgt | ctagccaagg | agttggcgcg | cggggccgcg | 120 |
| tcgacccaag | tgtgcaccgg | cacagacatg | aagctgcggc | tccctgccag | tcccagagacc | 180 |
| cacctggaca | tgtcccgcca | cctctaccag | ggctgccagg | tgggtgcagg | aaacctggaa | 240 |
| ctcacctacc | tgcccaccaa | tgccagcctg | tccttcctgc | aggatatcca | ggaggtgcag | 300 |
| ggctacgtgc | tcctcgtctc | caaccaagtg | aggcaggtcc | cactgcagag | gctgcggatt | 360 |
| gtgcgaggca | cccagctctt | tgaggacaac | tatgccctgg | ccgtgctaga | caatggagac | 420 |
| ccgctgaaca | ataccacccc | tgtcacaggg | gcctccccag | gaggcctgcg | ggagctgcag | 480 |
| cttcgaagcc | tcacagagat | cttgaaagga | ggggtcttga | tccagcgga | ccccagctc | 540 |
| tgctaccagg | acacgatttt | gtggaaggac | atcttcacac | agaacaacca | gctggctctc | 600 |
| acactgatag | acaccaaccg | ctctcggggc | tgccacccct | gttctccgat | gtgtaagggc | 660 |
| tcccgtgctg | ggggagagag | ttctgaggat | tgtcagagcc | tgacgcgcac | tgtctgtgcc | 720 |
| ggtggctgtg | cccgtgcaa | ggggccactg | cccactgact | gctgccatga | gcagtgtgct | 780 |
| gccggctgca | cgggccccaa | gcactctgac | tgcttgccct | gcctccactt | caaccacagt | 840 |
| ggcatctgtg | agctgcactg | cccagccctg | gtcacctaca | acacagacac | gtttgagtcc | 900 |
| atgcccaatc | ccgagggccg | gtatacattc | ggcgccagct | gtgtgactgc | ctgtccctac | 960 |
| aactaccttt | ctacggacgt | gggatccggc | gctgggggca | tggtccacca | caggcacccg | 1020 |
| agctcatcta | ccaggagtgg | cgggtggggc | ctgacactag | ggctggagcc | ctctgaagag | 1080 |
| gaggccccca | ggtctccact | ggcaccctcc | gaaggggctg | gctccgatgt | atttgatggt | 1140 |
| gacctgggaa | tgggggcagc | caaggggctg | caaagcctcc | ccacacatga | ccccagccct | 1200 |
| ctacagcggg | acagtggagg | ccccacagta | cccctgccct | ctgagactga | tggtactcgt | 1260 |
| gccccctga | cctgcagccc | ccagcctgaa | tatgtgaacc | agccagatgt | tcggccccag | 1320 |
| cccccttcgc | cccagagagg | ccctctgcct | gctgcccgac | ctgctggtgc | cactctggaa | 1380 |
| agggccaaag | ctctctcccc | agggaagaat | ggggctcgtc | aagacgtttt | tgctttggg | 1440 |
| ggtgccgtgg | agaaccccga | gtacttgaca | ccccagggag | gagctgcccc | tcagccccac | 1500 |
| cctcctcctg | ccttcagccc | agccttcgac | aacctctatt | actgggacca | ggaccacca | 1560 |
| gagcgggggg | ctccacccag | caccttcaaa | gggacaccta | cggcagagaa | cccagagtac | 1620 |
| ctgggtctgg | acgtgccagc | ggcgcacccc | gcccgtcgc | ccagccccag | cacacagccc | 1680 |
| tgggagcatg | tgaatgccat | ccaggaggcc | cggcgtctcc | tgaacctgag | tagagacact | 1740 |
| gctgctgaga | tgaatgaaac | agtagaagtc | atctcagaaa | tgtttgacct | ccaggagccg | 1800 |
| acctgcctac | agaccgcctt | ggagctgtac | aagcaggggc | tgccggggcag | cctcaccaag | 1860 |
| ctcaaggggc | ccttgaccat | gatggccagc | cactacaaac | agcactgccc | tccaaccccg | 1920 |
| gaaacttcct | gtgcaaccca | gattatcacc | tttgaaagtt | tcaaagagaa | cctgaaggac | 1980 |
| tttctgcttg | tcctcccttt | tgactgctgg | gagccagtcc | aggagggcgc | gccacccccg | 2040 |
| ccggcggccg | cacatcacca | tcaccatcac | | | | 2070 |

<210> 8

<211> 1692

<212> DNA

<213> Artificial Sequence

<220>

<223> HER500* construct

<400> 8

| | | | | | | |
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| atgagagctg | cacccctcct | cctggccagg | gcagcaagcc | ttagccttgg | cttcttggtt | 60 |
| ctgctttttt | tctggctaga | ccgaagtgt | ctagccaagg | agttggcgcg | cggggccgcg | 120 |
| tcgacccaag | tgtgcaccgg | cacagacatg | aagctgcggc | tccctgccag | tcccagagacc | 180 |
| cacctggaca | tgtcccgcca | cctctaccag | ggctgccagg | tgggtgcagg | aaacctggaa | 240 |
| ctcacctacc | tgcccaccaa | tgccagcctg | tccttcctgc | aggatatcca | ggaggtgcag | 300 |
| ggctacgtgc | tcctcgtctc | caaccaagtg | aggcaggtcc | cactgcagag | gctgcggatt | 360 |
| gtgcgaggca | cccagctctt | tgaggacaac | tatgccctgg | ccgtgctaga | caatggagac | 420 |
| ccgctgaaca | ataccacccc | tgtcacaggg | gcctccccag | gaggcctgcg | ggagctgcag | 480 |
| cttcgaagcc | tcacagagat | cttgaaagga | ggggtcttga | tccagcgga | ccccagctc | 540 |
| tgctaccagg | acacgatttt | gtggaaggac | atcttcacac | agaacaacca | gctggctctc | 600 |
| acactgatag | acaccaaccg | ctctcggggc | tgccacccct | gttctccgat | gtgtaagggc | 660 |
| tcccgtgctg | ggggagagag | ttctgaggat | tgtcagagcc | tgacgcgcac | tgtctgtgcc | 720 |
| ggtggctgtg | cccgtgcaa | ggggccactg | cccactgact | gctgccatga | gcagtgtgct | 780 |
| gccggctgca | cgggccccaa | gcactctgac | tgcttgccct | gcctccactt | caaccacagt | 840 |
| ggcatctgtg | agctgcactg | cccagccctg | gtcacctaca | acacagacac | gtttgagtcc | 900 |
| atgcccaatc | ccgagggccg | gtatacattc | ggcgccagct | gtgtgactgc | ctgtccctac | 960 |
| aactaccttt | ctacggacgt | gggatccgct | agcatcatta | atttcgagaa | gttggggcgt | 1020 |

| | | | | | | |
|------------|------------|------------|-------------|------------|------------|------|
| gggggcatgg | tccaccacag | gcaccgcagc | tcattctacca | ggagtggcgg | tggggacctg | 1080 |
| acactagggc | tggagccctc | tgaagaggag | gccccaggt | ctccactggc | accctccgaa | 1140 |
| ggggctggct | ccgatgtatt | tgatggtgac | ctgggaatgg | gggcagccaa | ggggctgcaa | 1200 |
| agcctcccca | cacatgaccc | cagccctcta | cagcggtaga | gtgaggaccc | cacagtaccc | 1260 |
| ctgccctctg | agactgatgg | ctacgttgcc | cccctgacct | gcagccccc | gcctgaatat | 1320 |
| gtgaaccagc | cagatgttcg | gccccagccc | ccttcgcccc | gagagggccc | tctgcctgct | 1380 |
| gcccgcctg | ctggtgccac | tctggaaagg | gccaaagactc | tctccccagg | gaagaatggg | 1440 |
| gtcgtcaaag | acgtttttgc | ctttgggggt | gccgtggaga | accccagta | cttgacaccc | 1500 |
| cagggaggag | ctgcccctca | gccccaccct | cctcctgcct | tcagcccagc | cttcgacaac | 1560 |
| ctctattact | gggaccagga | cccaccagag | cggggggctc | caccacgac | cttcaaaggg | 1620 |
| acacctacgg | cagagaaccc | agagtacctg | ggtctggacg | tgccagcggc | cgcacatcac | 1680 |
| catcaccatc | ac | | | | | 1692 |

<210> 9

<211> 2091

<212> DNA

<213> Artificial Sequence

<220>

<223> HER500*-rGM-CSF construct

<400> 9

| | | | | | | |
|------------|------------|------------|-------------|------------|-------------|------|
| atgagagctg | cacccctcct | cctggccagg | gcagcaagcc | ttagccttgg | cttcttgttt | 60 |
| ctgctttttt | tctggctaga | ccgaagtgt | ctagccaagg | agttggcgcg | cggggccgcg | 120 |
| tcgaccaag | tgtgcaccgg | cacagacatg | aagctgcggc | tccctgccag | tcccagagacc | 180 |
| cacctggaca | tgctccgcca | cctctaccag | ggctgccagg | tgggtgcagg | aaacctggaa | 240 |
| ctcacctacc | tgcccaacca | tgccagcctg | tccttcctgc | aggatatcca | ggaggtgcag | 300 |
| ggctacgtgc | tcacgtctca | caaccaagt | aggcaggctc | cactgcagag | gctgcggatt | 360 |
| gtgcgaggca | cccagctctt | tgaggacaac | tatgccttgg | ccgtgctaga | caatggagac | 420 |
| ccgctgaaca | ataccaaccc | tgtcacagg | gcctcccag | gaggcctgcg | ggagctgcag | 480 |
| cttcgaagcc | tcacagagat | cttgaaggga | ggggtcttga | tccagcggaa | ccccagctc | 540 |
| tgctaccagg | acacgatatt | gtggaaggac | atcttccaca | agaacaacca | gctggctctc | 600 |
| acactgatag | acaccaaccg | ctctcgggcc | tgccaccctt | gttctccgat | gtgtaagggc | 660 |
| tcccgtctgt | ggggagagag | ttctgaggat | tgctcagagc | tgacgcgcac | tgtctgtgcc | 720 |
| ggtggctgtg | cccgtctgca | ggggccactg | cccactgact | gctgccatga | gcagtgtgct | 780 |
| gccggctgca | cgggccccaa | gcactctgac | tgcttgccct | gcctccactt | caaccacagt | 840 |
| ggcactgtgt | agctgcactg | cccagccctg | gtcacctaca | acacagacac | gtttgagtcc | 900 |
| atgcccaatc | ccgagggccg | gtatacatte | ggcgccagct | gtgtgactgc | ctgtccctac | 960 |
| aactaccttt | ctacggacgt | gggatccgct | agcatcatta | atttcgagaa | gttgggcgct | 1020 |
| gggggcatgg | tccaccacag | gcaccgcagc | tcattctacca | ggagtggcgg | tggggacctg | 1080 |
| acactagggc | tggagccctc | tgaagaggag | gccccaggt | ctccactggc | accctccgaa | 1140 |
| ggggctggct | ccgatgtatt | tgatggtgac | ctgggaatgg | gggcagccaa | ggggctgcaa | 1200 |
| agcctcccca | cacatgaccc | cagccctcta | cagcggtaga | gtgaggaccc | cacagtaccc | 1260 |
| ctgccctctg | agactgatgg | ctacgttgcc | cccctgacct | gcagccccc | gcctgaatat | 1320 |
| gtgaaccagc | cagatgttcg | gccccagccc | ccttcgcccc | gagagggccc | tctgcctgct | 1380 |
| gcccgcctg | ctggtgccac | tctggaaagg | gccaaagactc | tctccccagg | gaagaatggg | 1440 |
| gtcgtcaaag | acgtttttgc | ctttgggggt | gccgtggaga | accccagta | cttgacaccc | 1500 |
| cagggaggag | ctgcccctca | gccccaccct | cctcctgcct | tcagcccagc | cttcgacaac | 1560 |
| ctctattact | gggaccagga | cccaccagag | cggggggctc | caccacgac | cttcaaaggg | 1620 |
| acacctacgg | cagagaaccc | agagtacctg | ggtctggacg | tgccagcggc | cgccccacc | 1680 |
| cgtcacccca | accctgtcac | ccggccctgg | aagcatgtag | atgccatcaa | agaagctctg | 1740 |
| agcctcctaa | atgacatgcg | tgctctggag | aacgaaaaga | acgaagacgt | agacatcatc | 1800 |
| tctaagtgt | tctccatcca | gaggccgaca | tgtgtgcaga | cccgcctgaa | gctatacaag | 1860 |
| cagggtctac | ggggaacct | caccaaactc | aatggcgccct | tgacctgat | agccagccac | 1920 |
| taccagacga | actgccctcc | aaccccggaa | actgactgtg | aaatagaagt | caccaccttt | 1980 |
| gaggatttca | taaagaacct | taaaggcttt | ctgtttgata | tcccttttga | ctgctggaag | 2040 |
| ccggtccaga | aaggcgcgcc | accccccgcc | gcgcatacc | atcacatca | c | 2091 |

<210> 10

<211> 1437

<212> DNA

<213> Artificial Sequence

<220>

<223> HER300*rGM-CSF construct

<400> 10

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atgagagctg caccctcct cctggccagg gcagcaagcc ttagccttgg cttcttgttt    60
ctgctttttt tctggctaga ccgaagtgtg ctagccaagg agttggcgcg cggggccgcg    120
tcgacccaag tgtgcaccgg cacagacatg aagctgcggc tccctgccag tcccagagacc    180
cacctggaca tgctccgcca cctctaccag ggctgccagg tgggtgcagg aaacctggaa    240
ctcacctacc tgcccaccaa tgccagcctg tccttcctgc aggatatcca ggaggtgcag    300
ggctacgtgc tcatcgctca caaccaagtg aggcagggtc cactgcagag gctgcggatt    360
gtgcgaggca ccagctctt tgaggacaac tatgccctgg ccgtgctaga caatggagac    420
ccgctgaaca ataccacccc tgtcacaggg gcctccccag gaggcctgcy ggagctgcag    480
cttcgaagcc tcacagagat cttgaaagga ggggtcttga tccagcgga cccccagctc    540
tgctaccagg acacgatttt gtggaaggac atcttcaca agaacaacca gctggctctc    600
acactgatag acaccaaccg ctctcggggc tgccaccctt gttctccgat gtgtaagggc    660
tcccgtgct ggggagagag ttctgaggat tgtcagagcc tgacgcgcac tgtctgtgcc    720
ggtggctgtg cccgctgcaa ggggccactg cccactgact gctgccatga gcagtgtgct    780
gccggctgca cgggcccacaa gcactctgac tgcttggcct gcctccactt caaccacagt    840
ggcatctgtg agctgcactg cccagccctg gtacacctac acacagacac gtttgagtcc    900
atgcccaatc ccgagggccg gtatacatlc ggcgccagct gtgtgactgc ctgtccctac    960
aactaccttt ctacggacgt ggatccgct agcatcatta atttcgagaa gttggccgcc   1020
cccacccgct caccacccc tgtcacccgg ccctggaagc atgtagatgc catcaaagaa   1080
gctctgagcc tcctaaatga catgcgtgct ctggagaacg aaaagaacga agacgtagac   1140
atcatctcta atgagttctc catccagagg ccgacatgtg tgcagaccg cctgaagcta   1200
tacaagcagg gtctacgggg caacctcacc aaactcaatg gcgccttgac catgatagcc   1260
agccactacc agacgaactg ccctccaacc ccgaaactg actgtgaaat agaagtcacc   1320
acctttgagg atttcataaa gaaccttaaa ggctttctgt ttgatatccc ttttgactgc   1380
tggaagccgg tccagaaagg cgcgccacc cgcggcgcg atcaccatca ccatcac    1437

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<210> 11

<211> 32

<212> PRT

<213> Homo sapiens

<400> 11

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Met Arg Ala Ala Pro Leu Leu Leu Ala Arg Ala Ala Ser Leu Ser Leu
 1             5             10             15
Gly Phe Leu Phe Leu Leu Phe Phe Trp Leu Asp Arg Ser Val Leu Ala
          20          25          30

```

<210> 12

<211> 3

<212> PRT

<213> Homo sapiens

<400> 12

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Lys Glu Leu
 1

```

<210> 13

<211> 3

<212> PRT

<213> Homo sapiens

<400> 13

```

Gly Ala Ala
 1

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<210> 14

<211> 2

<212> PRT

<213> Artificial Sequence

<220>

<223> linker

<400> 14

Ala Arg

1

<210> 15

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> C-terminal sequence of HER500 and HER500* constructs

<400> 15

Ala Ala Ala His His His His His His

1

5

<210> 16

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> C-terminal sequence of HER500-hGM-CSF construct

<400> 16

Gly Ala Pro Pro Pro Pro Ala Ala Ala His His His His His His

1

5

10

15

<210> 17

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> C-terminal sequence of HER500* and HER300* rat GM-CSF constructs

<400> 17

Gly Ala Pro Pro Pro Pro Ala His His His His His His

1

5

10

<210> 18

<211> 127

<212> PRT

<213> Homo sapiens

<400> 18

Ala Pro Ala Arg Ser Pro Ser Pro Ser Thr Gln Pro Trp Glu His Val

1

5

10

15

Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser Arg Asp Thr

20

25

30

Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu Met Phe Asp

35

40

45

Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys Gln

50

55

60

Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu Thr Met Met

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 65 | | | | | 70 | | | | | 75 | | | | 80 | |
| Ala | Ser | His | Tyr | Lys | Gln | His | Cys | Pro | Pro | Thr | Pro | Glu | Thr | Ser | Cys |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Ala | Thr | Gln | Ile | Ile | Thr | Phe | Glu | Ser | Phe | Lys | Glu | Asn | Leu | Lys | Asp |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Phe | Leu | Leu | Val | Ile | Pro | Phe | Asp | Cys | Trp | Glu | Pro | Val | Gln | Glu | |
| | | 115 | | | | | 120 | | | | | 125 | | | |

<210> 19

<211> 381

<212> DNA

<213> Homo sapiens

<400> 19

| | | | | | | |
|------------|------------|------------|------------|------------|------------|-----|
| gcacccgccc | gctcgcccag | ccccagcacg | cagccctggg | agcatgtgaa | tgccatccag | 60 |
| gagggccggc | gtctcctgaa | cctgagtaga | gacactgctg | ctgagatgaa | tgaaacagta | 120 |
| gaagtcacat | cagaaatgtt | tgacctccag | gagccgacct | gcctacagac | ccgcctggag | 180 |
| ctgtacaagc | agggcctgcg | gggcagcctc | accaagctca | agggccctt | gaccatgatg | 240 |
| gccagccact | acaagcagca | ctgccctcca | accccgga | cttcctgtgc | aaccagact | 300 |
| atcacctttg | aaagtttcaa | agagaacctg | aaggactttc | tgcttgtcat | cccctttgac | 360 |
| tgctgggagc | cagtccagga | g | | | | 381 |

<210> 20

<211> 127

<212> PRT

<213> Rattus norvegicus

<400> 20

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Pro | Thr | Arg | Ser | Pro | Asn | Pro | Val | Thr | Arg | Pro | Trp | Lys | His | Val |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Asp | Ala | Ile | Lys | Glu | Ala | Leu | Ser | Leu | Leu | Asn | Asp | Met | Arg | Ala | Leu |
| | | | 20 | | | | | 25 | | | | 30 | | | |
| Glu | Asn | Glu | Lys | Asn | Glu | Asp | Val | Asp | Ile | Ile | Ser | Asn | Glu | Phe | Ser |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Ile | Gln | Arg | Pro | Thr | Cys | Val | Gln | Thr | Arg | Leu | Lys | Leu | Tyr | Lys | Gln |
| | | 50 | | | | 55 | | | | | 60 | | | | |
| Gly | Leu | Arg | Gly | Asn | Leu | Thr | Lys | Leu | Asn | Gly | Ala | Leu | Thr | Met | Ile |
| 65 | | | | 70 | | | | | 75 | | | | | 80 | |
| Ala | Ser | His | Tyr | Gln | Thr | Asn | Cys | Pro | Pro | Thr | Pro | Glu | Thr | Asp | Cys |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Glu | Ile | Glu | Val | Thr | Thr | Phe | Glu | Asp | Phe | Ile | Lys | Asn | Leu | Lys | Gly |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Phe | Leu | Phe | Asp | Ile | Pro | Phe | Asp | Cys | Trp | Lys | Pro | Val | Gln | Lys | |
| | | 115 | | | | | 120 | | | | | 125 | | | |

<210> 21

<211> 381

<212> DNA

<213> Rattus norvegicus

<400> 21

| | | | | | | |
|------------|-------------|------------|------------|------------|------------|-----|
| gcacccaccc | gctcacccaa | ccctgtcacc | cggccctgga | agcatgtaga | tgccatcaaa | 60 |
| gaagctctga | gcctcctaaa | tgacatgcgt | gctctggaga | acgaaaagaa | cgaagacgta | 120 |
| gacatcatct | ctaagtgagtt | ctccatccag | aggccgacat | gtgtgcagac | ccgcctgaag | 180 |
| ctatacaagc | agggctctacg | gggcaacctc | accaaactca | atggcgcctt | gaccatgata | 240 |
| gccagccact | accagacgaa | ctgccctcca | accccgga | ctgactgtga | aatagaagtc | 300 |
| accacctttg | aggatttcat | aaagaacctt | aaaggctttc | tgtttgatat | cccttttgac | 360 |
| tgctggaagc | cggtcagaa | a | | | | 381 |

<210> 22

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> OVA-derived immunodominant octapeptide

<400> 22

Ser Ile Ile Asn Phe Glu Lys Leu

1 5

<210> 23

<211> 289

<212> PRT

<213> Homo sapiens

<400> 23

Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys Leu Arg Leu Pro Ala
1 5 10 15Ser Pro Glu Thr His Leu Asp Met Leu Arg His Leu Tyr Gln Gly Cys
20 25 30Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr Leu Pro Thr Asn Ala
35 40 45Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val Gln Gly Tyr Val Leu
50 55 60Ile Ala His Asn Gln Val Arg Gln Val Pro Leu Gln Arg Leu Arg Ile
65 70 75 80Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr Ala Leu Ala Val Leu
85 90 95Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro Val Thr Gly Ala Ser
100 105 110Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser Leu Thr Glu Ile Leu
115 120 125Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln Leu Cys Tyr Gln Asp
130 135 140Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn Asn Gln Leu Ala Leu
145 150 155 160Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys His Pro Cys Ser Pro
165 170 175Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser Ser Glu Asp Cys Gln
180 185 190Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys Ala Arg Cys Lys Gly
195 200 205Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys Ala Ala Gly Cys Thr
210 215 220Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu His Phe Asn His Ser
225 230 235 240Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val Thr Tyr Asn Thr Asp
245 250 255Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg Tyr Thr Phe Gly Ala
260 265 270Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu Ser Thr Asp Val Gly
275 280 285

Ser

<210> 24

<211> 867

<212> DNA

<213> Homo sapiens

<400> 24

agcaccacaag tgtgcaccgg cacagacatg aagctgcccg tccctgccag tcccagagacc 60
cacctggaca tgctccgcca cctctaccag ggctgccagg tgggtgcagg aaacctggaa 120

```

ctcacctacc tgcccaccaa tgccagcctg tccctcctgc aggatatcca ggaggtgcag 180
ggctacgtgc tcatcgctca caaccaagtg aggcaggctc cactgcagag gctgcggatt 240
gtgcgaggca cccagctctt tgaggacaac tatgccctgg ccgtgctaga caatggagac 300
ccgctgaaca ataccacccc tgtcacaggg gcctccccag gaggcctgcg ggagctgcag 360
cttcgaagcc tcacagagat cttgaaagga ggggtcttga tccagcggaa cccccagctc 420
tgctaccagg acacgatttt gtggaaggac atcttcacac agaacaacca gctggctctc 480
aactgatag acaccaaccg ctctcgggcc tgccaccctt gttctccgat gtgtaagggc 540
tcccgtgct ggggagagag ttctgaggat tgtcagagcc tgacgcgcac tgtctgtgcc 600
ggtggctgtg cccgctgcaa ggggccactg cccactgact gctgccatga gcagtgtgct 660
gccggctgca cgggccccaa gcactctgac tgccctggcct gcctccactt caaccacagt 720
ggcatctgtg agctgcactg cccagccctg gtcacctaca acacagacac gtttgagtc 780
atgcccaatc ccgagggccg gtatacatc ggcgcagct gtgtgactgc ctgtccctac 840
aactaccttt ctacggacgt gggatcc 867

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<210> 25

<211> 217

<212> PRT

<213> Homo sapiens

<400> 25

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Gly Ala Gly Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg
1          5          10          15
Ser Gly Gly Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu
20          25          30
Ala Pro Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser Asp Val
35          40          45
Phe Asp Gly Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln Ser Leu
50          55          60
Pro Thr His Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr
65          70          75          80
Val Pro Leu Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu Thr Cys
85          90          95
Ser Pro Gln Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro Gln Pro
100         105         110
Pro Ser Pro Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala Gly Ala
115         120         125
Thr Leu Glu Arg Ala Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val
130         135         140
Lys Asp Val Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu
145         150         155         160
Thr Pro Gln Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro Ala Phe
165         170         175
Ser Pro Ala Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu
180         185         190
Arg Gly Ala Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu Asn
195         200         205
Pro Glu Tyr Leu Gly Leu Asp Val Pro
210         215

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<210> 26

<211> 651

<212> DNA

<213> Homo sapiens

<400> 26

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ggcgtgggg gcattggtcca ccacaggcac cgcagctcat ctaccaggag tggcgggtggg 60
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tccgaagggg ctggctccga tgtatttgat ggtgacctgg gaatgggggc agccaagggg 180
ctgcaaagcc tccccacaca tgacccacgc cctctacagc ggtacagtga ggacccaca 240
gtacccctgc cctctgagac tgatggctac gttgcccccc tgacctgcag ccccagcct 300
gaatatgtga accagccaga tgttcggccc cagccccctt cggcccgaga gggccctctg 360
cctgctgccc gacctgctgg tgccactctg gaaagggcca agactctctc cccagggaag 420

```

```

aatgggggtcg tcaaagacgt ttttgccttt ggggggtgccg tggagaaccc cgagtacttg 480
acaccccagg gaggagctgc ccctcagccc caccctcctc ctgccttcag cccagccttc 540
gacaacctct attactggga ccaggaccca ccagagcggg gggctccacc cagcaccttc 600
aaagggacac ctacggcaga gaacccagag tacctgggtc tggacgtgcc a 651

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<210> 27

<211> 397

<212> PRT

<213> Artificial Sequence

<220>

<223> NY-ESO-IC tumor antigen

<400> 27

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Met Gln Ala Glu Gly Arg Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp
 1           5           10          15
Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly
          20          25          30
Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala
          35          40          45
Gly Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro
          50          55          60
His Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Cys Arg Cys Gly Ala
65          70          75          80
Arg Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe
          85          90          95
Ala Thr Pro Met Glu Ala Glu Leu Ala Arg Arg Ser Leu Ala Gln Asp
          100         105         110
Ala Pro Pro Leu Pro Val Pro Gly Val Leu Leu Lys Glu Phe Thr Val
          115         120         125
Ser Gly Asn Ile Leu Thr Ile Arg Leu Thr Ala Ala Asp His Arg Gln
          130         135         140
Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met
145         150         155         160
Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Pro Pro Ser
          165         170         175
Gly Gln Arg Arg Gly Ala Gly Gly Met Val His His Arg His Arg Ser
          180         185         190
Ser Ser Thr Arg Ser Gly Gly Gly Asp Leu Thr Leu Gly Leu Glu Pro
          195         200         205
Ser Glu Glu Glu Ala Pro Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala
          210         215         220
Gly Ser Asp Val Phe Asp Gly Asp Leu Gly Met Gly Ala Ala Lys Gly
225         230         235         240
Leu Gln Ser Leu Pro Thr His Asp Pro Ser Pro Leu Gln Arg Tyr Ser
          245         250         255
Glu Asp Pro Thr Val Pro Leu Pro Ser Glu Thr Asp Gly Tyr Val Ala
          260         265         270
Pro Leu Thr Cys Ser Pro Gln Pro Glu Tyr Val Asn Gln Pro Asp Val
          275         280         285
Arg Pro Gln Pro Pro Ser Pro Arg Glu Gly Pro Leu Pro Ala Ala Arg
          290         295         300
Pro Ala Gly Ala Thr Leu Glu Arg Ala Lys Thr Leu Ser Pro Gly Lys
305         310         315         320
Asn Gly Val Val Lys Asp Val Phe Ala Phe Gly Gly Ala Val Glu Asn
          325         330         335
Pro Glu Tyr Leu Thr Pro Gln Gly Gly Ala Ala Pro Gln Pro His Pro
          340         345         350
Pro Pro Ala Phe Ser Pro Ala Phe Asp Asn Leu Tyr Tyr Trp Asp Gln
          355         360         365
Asp Pro Pro Glu Arg Gly Ala Pro Pro Ser Thr Phe Lys Gly Thr Pro
          370         375         380

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Thr Ala Glu Asn Pro Glu Tyr Leu Gly Leu Asp Val Pro
 385 390 395

<210> 28
 <211> 1191
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> NY-ESO-IC tumor antigen

<400> 28
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 cctggcattc ctgatggccc agggggcaat gctggcggcc caggagaggc ggggtgccacg 120
 ggcggcagag gtccccgggg cgcaggggca gcaaggcct cggggccggg aggaggcgcc 180
 ccgcgggggtc cgcattggcg cgcggcttca gggctgaatg gatgctgcag atgcggggcc 240
 agggggccgg agagccgcct gcttgagttc tacctcgcca tgcctttcgc gacacccatg 300
 gaagcagagc tggcccgcag gagcctggcc caggatgccc caccgcttcc cgtgccaggg 360
 gtgcttctga aggagttcac tgtgtccggc aacatactga ctatccgact gactgctgca 420
 gaccaccgcc aactgcagct ctccatcagc tcctgtctcc agcagcttcc cctgttgatg 480
 tggatcacgc agtgctttct gccogtgttt ttggctcagc ctccctcagg gcagaggcgc 540
 ggcgtggggg gcatgggtcca ccacaggcac cgcagctcat ctaccaggag tggcgggtggg 600
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 tccgaagggg ctggctccga tgtatttgat ggtgacctgg gaatgggggc agccaagggg 720
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 65 70 75 80
 Gly Glu Tyr Glu Trp Glu Tyr Asp Glu Glu Glu Lys Asn Gln Leu
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 100 105 110
 Asn Cys His Val Asp Leu Ile Arg Leu Leu Arg Leu Glu Gly Glu Leu
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 145 150 155 160

Gln Asp Gly Leu Asp Arg Glu His Val Tyr Asp Leu Phe Glu Lys Ala
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 Val Lys Asp Tyr Ile Cys Pro Asn Ile Trp Leu Glu Tyr Gly Gln Tyr
 180 185 190
 Ser Val Gly Gly Ile Gly Gln Lys Gly Gly Leu Glu Lys Val Arg Ser
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 260 265 270
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| | | | | | | | | | | | | | | | | | |
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| Val | Asp | Val | Glu | Pro | Pro | Ser | Lys | Gln | Lys | Glu | Lys | Ala | Ala | Ser | Leu | | |
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| Lys | Leu | Arg | Pro | Leu | Phe | Glu | Ala | Cys | Gly | Glu | Val | Val | Gln | Ile | Arg | | |
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| | | | 740 | | | | | 745 | | | | | 750 | | | | |
| Phe | Lys | Glu | Glu | Lys | Ser | Ala | Leu | Gln | Ala | Leu | Glu | Met | Asp | Arg | Lys | | |
| | | | 755 | | | | 760 | | | | | 765 | | | | | |
| Ser | Val | Glu | Gly | Arg | Pro | Met | Phe | Val | Ser | Pro | Cys | Val | Asp | Lys | Ser | | |
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| Lys | Asn | Pro | Asp | Phe | Lys | Val | Phe | Arg | Tyr | Ser | Thr | Ser | Leu | Glu | Lys | | |
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| His | Lys | Leu | Phe | Ile | Ser | Gly | Leu | Pro | Phe | Ser | Cys | Thr | Lys | Glu | Glu | | |
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| Leu | Glu | Glu | Ile | Cys | Lys | Ala | His | Gly | Thr | Val | Lys | Asp | Leu | Arg | Leu | | |
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| Arg | Lys | Val | Pro | Glu | Lys | Pro | Glu | Thr | Arg | Lys | Ala | Pro | Gly | Gly | Pro | | |
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| Met | Leu | Leu | Pro | Gln | Thr | Tyr | Gly | Ala | Arg | Gly | Lys | Gly | Arg | Thr | Gln | | |
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| Gln | Ala | Glu | Asn | Gly | Pro | Ala | Ala | Ala | Pro | Ala | Val | Ala | Ala | Pro | Ala | | |
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| | | | 1090 | | | 1095 | | | | | 1100 | | | | | | |
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| | | | | 1110 | | | | | 1115 | | | | | | 1120 | | |
| Tyr | Leu | Thr | Pro | Gln | Gly | Gly | Ala | Ala | Pro | Gln | Pro | His | Pro | Pro | Pro | | |
| | | | | 1125 | | | | | 1130 | | | | | 1135 | | | |
| Ala | Phe | Ser | Pro | Ala | Phe | Asp | Asn | Leu | Tyr | Tyr | Trp | Asp | Gln | Asp | Pro | | |
| | | | 1140 | | | | | 1145 | | | | | 1150 | | | | |

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| gccttcgaca | acctctatta | ctgggaccag | gacccaccag | agcggggggc | tccaccagc | 3480 |
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